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# IMPLICAÇÕES DA DIVERSIDADE GENÉTICA INTRA-HOSPEDEIRO DO DENGUE VÍRUS SOROTIPO 2 NA PATOGÊNESE DA DENGUE

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**“Nada em biologia faz sentido**
**exceto à luz da evolução”**
**Theodosius Dobzhansky 1973.**

RESUMO

## IMPLICAÇÕES DA DIVERSIDADE GENÉTICA INTRA-HOSPEDEIRO DO DENGUE VÍRUS SOROTIPO 2 NA PATOGÊNESE DA DENGUE

A infecção pelo vírus da dengue (DENV) pode variar de infecção assintomática a uma doença aguda debilitante e potencialmente fatal em hospedeiros humanos. A teoria da amplificação dependente de anticorpos (ADE - do inglês *antibody dependent enhancement*)) explica por que certos casos progridem para gravidade; no entanto, ainda é controverso, uma vez que muitos casos de dengue hemorrágico ocorrem em infecções primárias, indicando que o ADE não é essencial para o desenvolvimento de uma clínica de maior gravidade. Vários estudos apontam os fatores virais como responsáveis pelo aumento da virulência e da patogênese da doença. Os DENVs são vírus de RNA que no hospedeiro existem como subpopulações geneticamente diversas devido à sua replicação propensa a erros. Acredita-se que a diversidade genética intra-hospedeiro facilite a adaptação de arbovírus a diferentes ambientes e hospedeiros, e esta, pode estar relacionada à patogênese viral. Com o objetivo de aumentar o conhecimento acerca dos determinantes virais envolvidos na patogênese grave da dengue, analisamos a diversidade genética intra-hospedeiro do DENV-2 em 68 pacientes infectados classificados clinicamente como dengue (n=31), dengue com sinais de alarme (n=19) e dengue grave (n=18). Ao contrário dos estudos anteriores de diversidade intra-hospedeiro de DENV, cujas abordagens empregaram PCR, aqui realizamos o sequenciamento massivo do genoma viral inteiro a partir de amostras clínicas com uma abordagem livre de amplificação por PCR, representando o cenário mais próximo de diversidade intra-hospedeiro. Diferenças marcantes foram detectadas na estrutura da população viral entre as três categorias clínicas, as quais parecem ser consequência principalmente dos diferentes tempos de infecção e pressões de seleção, em vez de estarem associadas ao próprio desfecho clínico. A diversidade no gene NS2B, no entanto, mostrou-se limitada, independentemente da apresentação clínica e do tempo de infecção, tornando à proteína um alvo atraente para o desenho inteligente de drogas. Além disso, dentre as 1.474 variantes diferentes que foram achadas de forma consistente entre as amostras, junto com outras 1.232 variantes únicas, um conjunto de 141 mutações relevantes distribuídas por todo o genoma viral se destacou por sua possível associação com os desfechos clínicos dos pacientes. Portanto, empregamos modelagem molecular para avaliar seu potencial efeito estrutural e/ou funcional nas proteínas virais e nas estruturas secundárias do RNA. No geral, os resultados mostraram que as variantes disruptivas foram identificadas principalmente entre os casos de dengue clássico, enquanto as variantes potenciais de escape imunológico foram principalmente associadas aos casos de maior gravidade, em linha com os tempos de evolução intra-hospedeiro mais longos destes últimos. No entanto, estudos funcionais seriam necessários para confirmar nossos achados. Além disso, várias mutações foram localizadas em regiões de superfície de proteínas, o que exigiria mais pesquisas para desvendar possíveis interações complexas entre proteínas virais e do hospedeiro. A presente análise fornece novas informações sobre as implicações da diversidade genética intra-hospedeiro do DENV-2, contribuindo para o conhecimento dos fatores virais possivelmente envolvidos em sua patogênese no hospedeiro humano.

ABSTRACT

**IMPLICATIONS OF THE DENGUE VIRUS SEROTYPE 2 GENETIC INTRAHOST DIVERSITY IN THE PATHOGENESIS OF DENGUE**

Dengue virus (DENV) infection can range from asymptomatic infection to a debilitating and potentially life-threatening acute disease in human hosts. Antibody-dependent enhancement (ADE) theory does explain why certain cases do progress to severity; however, it is still controversial since many hemorrhagic dengue cases occurred in primary DENV infections, indicating that ADE is not essential to disease severity. Several studies have pointed out viral factors as responsible for increased virulence and disease pathogenesis. DENVs are RNA viruses that within the host exist as genetically diverse subpopulations due to their error-prone nucleic acid replication. Intra-host genetic diversity is thought to facilitate arbovirus adaptation to changing environments and hosts, and it may also be linked to viral pathogenesis. Intending to shed light on the viral determinants for severe dengue pathogenesis, we sought to analyze the DENV-2 intrahost genetic diversity in 68 patient cases clinically classified as dengue fever (n = 31), dengue with warning signs (n = 19), and severe dengue (n = 18). Unlike previous DENV intrahost diversity studies whose approaches employed PCR, here we performed viral whole-genome deep sequencing from clinical samples with an amplicon-free approach, representing the real intrahost diversity scenario. Striking differences were detected in the viral population structure between the three clinical categories, which appear to be driven mainly by different infection times and selection pressures rather than being linked with the clinical outcome itself. Diversity in the NS2B gene, however, showed to be constrained, irrespective of clinical outcome and infection time, making its protein-product an attractive target for intelligent drug design. Moreover, among the 1474 different variants that were consistently repeated among samples plus the 1232 unique variants, a set of 141 relevant mutations distributed throughout the entire viral genome stood out for its possible association with patients' clinical outcomes. Therefore, we employed molecular modeling to assess their potential structural and/or functional effect on the viral proteins/RNA secondary structures. Overall, the results showed that disruptive variants were primarily identified among DF cases. In contrast, potentially immune-escape variants mainly were associated with WS+SD cases, in line with the latter's longer intrahost evolution times. Functional studies would be needed to confirm our findings. Furthermore, several mutations were located on proteins-surface regions, which would require further research to disentangle possible complex interactions between viral and host proteins. The present analysis provides new information about the implications of the intrahost genetic diversity of DENV-2, contributing to the knowledge about the viral factors possibly involved in its pathogenesis within the human host.

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## LISTA DE SIGLAS E ABREVIATURAS

aa – amino ácido.

Acs – anticorpos.

ANOVA – análise da variância.

BR – Brasil.

C – proteína do capsídeo.

Cc M – concentração molar.

cDNA – ácido desoxirribonucleico complementar, do inglês “complementary deoxyribonucleic acid”.

CEP – comitê de ética em pesquisa.

D.C – depois de Cristo.

DENV – vírus dengue.

DENV-1, DENV-2, DENV-3, DENV-4 – vírus dengue sorotipo 1, 2, 3 e 4.

DF – dengue clássico, do inglês “dengue fever”.

DNA – ácido desoxirribonucleico, do inglês “deoxyribonucleic acid”.

dNTP – desoxiribonucleotideo trifosfato, do inglês “deoxynucleotide triphosphate”.

ELISA – ensaio de imunoabsorção enzimática, do inglês “Enzyme-Linked ImmunoSorbent Assay”.

E – proteína do envelope.

ES – Estado do Espírito Santo.

FUNED - Fundação Ezequiel Dias.

Gt – Genótipo

HCV – vírus da hepatite C.

HIV – vírus da imunodeficiência humana.

$H_2O$ – água.

IgG – imunoglobulina G.

IgM – imunoglobulina M.

IMT - Instituto de Medicina Tropical.

INF – interferon.

LACEN - Laboratório Central.

M – glicoproteína de membrana.

MG – Estado de Minas Gerais.

ML – máxima verossimilhança.

MS – Ministério de Saúde.

NGS – sequenciamento de nova geração, do inglês “next generation sequencing”.

NS – Substituição não sinônima.

nt – nucleotídeo

PCR – reação em cadeia da polimerase, do inglês “polymerase chain reaction”.

PDK – células de rim de cão, do inglês “primary dog kidney”.

UFP – unidade formadora de placa.

pH – coeficiente que indica o grau de acidez ou basicidade de uma solução aquosa.

PRI – infecção primária.

prM – glicoproteína de membrana imatura.

Q – “score” de qualidade.

qPCR – reação em cadeia da polimerase quantitativa, do inglês “quantitative polymerase chain reaction”.

RJ – Estado do Rio de Janeiro.

RNA – ácido ribonucleico, do inglês “ribonucleic acid”.

RT-PCR – transcrição reversa seguida da reação em cadeia da polimerase, do inglês “reverse transcription followed by polymerase chain reaction”.

SD – dengue grave, do inglês “severe dengue”.

sfRNA – RNAs curtos derivados da degradação do RNA genômico dos flavivírus, do inglês “short flavivirus RNA”.

SEC – infecção secundária.

SP – Estado de São Paulo.

iSNV – variante de nucleotídeo único, do inglês “intrahost single nucleotide variation”.

SS – Substituição sinônima.

SVS – Secretaria de vigilância em saúde.

UTR – região não codificante de proteínas, do inglês “untranslated region”.

WHO – Organização Mundial da Saúde, do inglês “World Health Organization”.

WS – dengue com sinais de alarme, do inglês “dengue with warning signs”.

# 1 INTRODUÇÃO

## 1.1 Breve descrição da doença

A Dengue é a arbovirose de maior importância epidemiológica no mundo devido ao seu alto índice de morbimortalidade. A doença causada por um dos quatro sorotipos do vírus dengue (DENV), DENV-1, DENV-2, DENV-3 e DENV-4, é endêmica em mais de 100 países e nos últimos 60 anos, vem progressivamente alcançando o perfil de pandemia global, sendo estimado o número de infecções anuais em torno de 390 milhões, dos quais 96 milhões manifestam ao menos algum sinal de gravidade (revisado em WHO, 2020). O homem é o único hospedeiro capaz de desenvolver a doença, a qual é transmitida por mosquitos do gênero *Aedes*, tendo o *Aedes aegypti* como a principal espécie vetorial na região das Américas (WHO, 2009).

## 1.2 Breve histórico

O termo “dengue” provavelmente originou-se na Espanha no início do século XIX, sendo um homônimo para a expressão de origem africana “Ki Denga Pepo”, ou “Denga”, cujo significado é “pancada ou golpe causado por um espírito mau que provocava um ataque doloroso”. Em 1828, a literatura médica inglesa adotou o termo durante uma epidemia, ocorrida no Caribe, de doença exantemática com artralgia (Halstead, 1980; Schatzmayr, 2008). Existem registros históricos da ocorrência de doença clinicamente compatível com a dengue na China publicados durante a Dinastia Jin, 265 – 420 D.C., sendo relatada formalmente nas Dinastias Tang, 610 D.C, e Sung do Norte, 992 D.C. A enfermidade foi chamada naquela época de “veneno da água”, já havendo a associação de insetos voadores com a água (Gubler, 1998; Weaver & Vasilakis, 2009). Surtos ocorridos nas Índias Francesas Ocidentais e no Panamá em 1635 e 1699, respectivamente, também foram relacionados à Dengue, porém os primeiros registros de grandes epidemias ocorreram nos anos de 1779 e 1780, atingindo os continentes da Ásia, África e América do Norte. Estes episódios importantes da dengue ocorreram, quase que simultaneamente em 1779, em Jakarta (Indonésia)**,** e Egito, e em 1780 na Filadélfia (Gubler, 1998; Halstead,1980). Em 1907, enquanto Ashburn e Craig desenvolviam uma série de experimentos para investigar a

febre da dengue (DF) nas Filipinas, encontraram um agente filtrável e infeccioso no sangue humano (Ashburn & Craig, 1907). A transmissão do DENV pelo mosquito *Aedes aegypti* foi primeiramente sugerida por Bancroft, em 1906, (Bancroft, 1906) e mais tarde confirmada por Cleland e colaboradores, em 1918, ao testarem, em voluntários humanos, a capacidade de transmissão do DENV pelos mosquitos *Culex fatigans* e *Ae. aegypti*, obtendo sucesso apenas com o último (Cleland et al., 1919).

O vírus da Dengue foi isolado em camundongos por Kimura e Hotta, em 1943, e por Sabin e Schelinger, em 1944, sendo neste caso, feito o isolamento das cepas Havaí e de Nova Guiné. Pesquisas realizadas por Sabin, durante a Segunda Guerra Mundial, forneceram a prova da existência de diferentes características antigênicas entre os DENV, sendo a cepa do Havaí caracterizada como sorotipo 1 e a cepa de Nova Guiné como sorotipo 2, as duas consideradas hoje protótipos (Kimura & Hotta, 1944; Sabin, 1952; Sabin & Schlesinger, 1945).

Os sorotipos 3 e 4 foram isolados a partir de pacientes com quadro grave de febre hemorrágica, durante epidemia ocorrida em Manila, nas Filipinas, em 1956, onde se registrou a co-circulação dos quatro sorotipos de Dengue. Foi sugerido que a co-circulação destes sorotipos proporcionou o surgimento de maior número de casos graves da doença (Hammond et al., 1960).

Provavelmente, os primeiros casos de infecção pelo vírus da Dengue surgiram na Ásia, onde os sorotipos DENV-1, 2 e 4 foram demonstrados em ciclos silvestres. A Segunda Guerra Mundial, favoreceu condições à expansão dos vetores da Dengue, tornando-a epidêmica e proporcionando o surgimento de formas clínicas graves da doença classificadas como: a Febre Hemorrágica por Dengue e a Síndrome do Choque por Dengue (Rudnick, 1986; Schatzmayr, 2008).

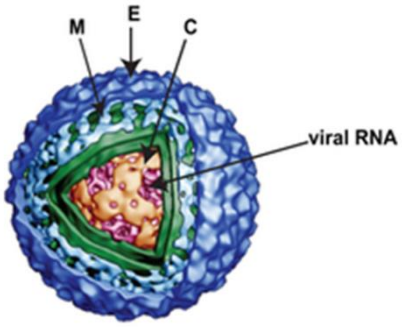
## 1.3 Vírus Dengue

O vírus Dengue (DENV) pertence à família *Flaviviridae,* gênero *Flavivirus*. Epidemiológicamente são classificados como Arbovírus, pois são transmitidos por artrópodes, sendo neste caso o mosquito vetor do gênero *Aedes* (Gubler, 2002).

### *1.3.1 Estrutura do vírus*

Trata-se de um vírus esférico e envelopado. O vírion maduro é caracterizado por uma superfície lisa com aproximadamente 50 nanômetros (nm) de diâmetro, enquanto o vírion imaturo tem 60 nm de diâmetro com uma superfície rugosa (Li et al., 2008). O capsídeo de simetria icosaédrica é composto por uma única proteína, a proteína do capsídeo, e é circundado por uma bicamada lipídica a qual se associam as proteínas virais da membrana e do envelope (figura 1.1) (Kuhn et al., 2002).

**Figura 1.1** Estrutura do vírus dengue



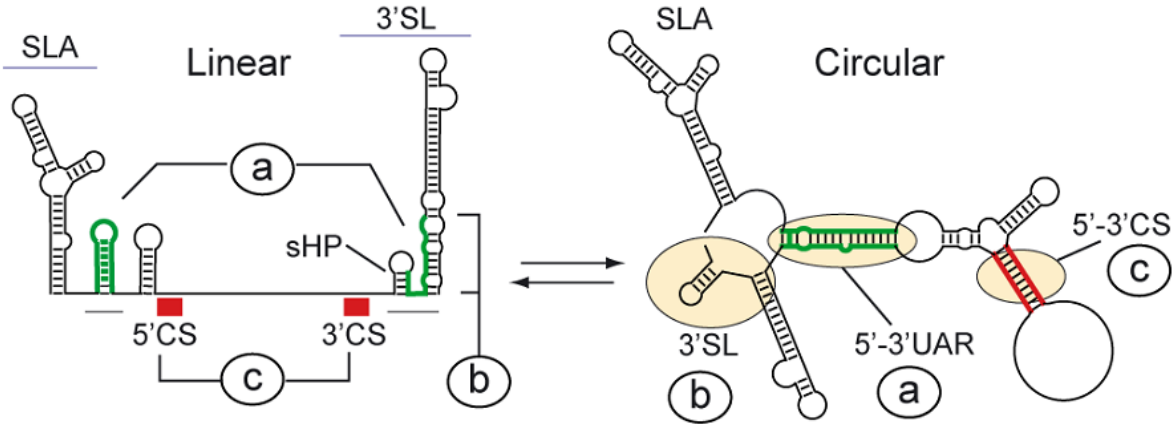

Fonte: imagem adaptada de Angel & Valle, 2013. M: glicoproteína da membrana; E: proteína do envelope; C: capsídeo.

### *1.3.2 Estrutura gênica*

O genoma do DENV é composto por uma fita simples de RNA de polaridade positiva, de aproximadamente 11 kilobases, contendo uma única fase de leitura aberta (ORF, do inglês “open reading frame”), flanqueado por duas regiões não codificantes (5'UTR e 3'UTR do inglês “untranslated region”) (Chambers et al., 1990). O genoma tem uma estrutura “cap” tipo 1 (m7GpppAmpN2) na extremidade 5' UTR e não possui cauda poli A na extremidade 3'UTR (Gebhard et al., 2011; Iglesias et al., 2011). O genoma do DENV funciona como uma molécula dinâmica, mudando dentro de um equilíbrio entre formas linear e circular. Uma série de sequencias complementares e estruturas secundarias funcionais do RNA existem na forma linear do genoma e se sobrepõem, atuando como elementos de ciclização e fornecendo um mecanismo para controlar as conformações do RNA viral durante a replicação (Figura 1.2) (Alvarez et

al., 2005; Villordo et al., 2010). A regulação das conformações do genoma também pode modular a eficiência da tradução viral (de Borba et al., 2019).

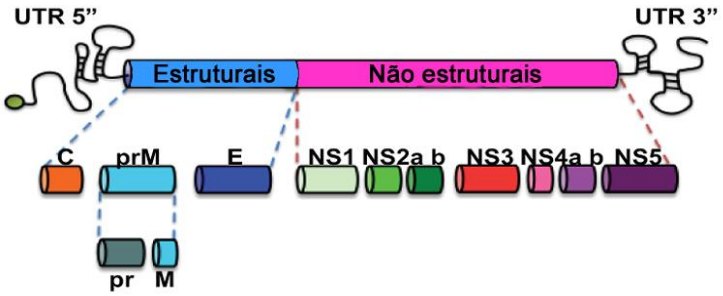
**Figura 1.2** Ciclização do genoma viral




Fonte: imagem adaptada de Gebhard et al., 2011. SLA, 3'SL: estruturas secundárias em forma de haste, do inglês "Stem Loop"; 5/3'CS: regiões complementares dentro do genoma viral, do inglês "complementary sequences"; 5/3'UAR: região do genoma viral que antecede ao códon de iniciação da tradução, do inglês "upstream AUG region".

A fase de leitura codifica para uma única poliproteína, a qual é clivada por proteases virais e celulares em múltiplos sítios, dando origem às proteínas estruturais (capsídeo, membrana e envelope) e às não estruturais (NS1, NS2A, NS2B, NS3, NS4A, NS4B e NS5) (Figura 1.3) (Chambers et al., 1990).

**Figura 1.3** Estrutura gênica do DENV



Fonte: imagem adaptada de Angel & Valle, 2013. UTR: região não codificante, do inglês “untranslated region”; C: capsídeo; M: glicoproteína de membrana; prM: forma imatura da glicoproteína de membrana; E: envelope; NS: proteína não estrutural.

#### *1.3.2.1 Proteínas estruturais*

A proteína do capsídeo (C) é crucial para a formação do nucleocapsídeo (proteína C + RNA genômico) durante os estágios primários da montagem do vírion (Murphy, 1980). Trata-se de um homodímero (114 aminoácidos cada unidade) que contém uma distribuição de carga assimétrica, apresentando sobre uma face da superfície do dímero uma região básica a partir da qual interage com o RNA genômico viral. A ligação do RNA viral ao capsídeo inicia uma agregação desta proteína associada à membrana conformando assim a partícula viral imatura e induzindo o seu brotamento no RE. Por outro lado, o achado desta proteína no núcleo celular leva à hipótese de que presumivelmente interage também com o RNA do hospedeiro, alterando o splicing e a transcrição do RNA, modificando a biogênese dos ribossomos e o transcriptoma do hospedeiro. Do lado oposto do dímero, uma região hidrofóbica interage com membrana. Tem se observado que prévio à encapsidação, os dímeros são armazenados em gotículas de lipídios. Essa interação é essencial para a produção eficiente de partículas virais (Sotcheff e Routh, 2020). Paralelamente às funções da proteína C, resulta importante destacar que na região genômica que codifica para a mesma existem também elementos chamados de 5’DAR (do inglês “downstream AUG region”, fazendo referência ao códon de iniciação da tradução), cHP (do inglês “hairpin”) e 5’CS (do inglês “cyclization sequence”), que estão envolvidos na replicação, tradução e ciclização do genoma viral, respectivamente (Alvarez et al., 2005; Clyde et al., 2008; Friebe et al., 2011).

A glicoproteína prM/M consiste em 166 aminoácidos, dos quais os 91 da região N-terminal (fragmento “pr”) são liberados após a clivagem pela furina celular, deixando apenas o ectodomínio (resíduos 92 a 130) e a região C-terminal transmembrana (resíduos 131 a 166) no vírion. O peptídeo pr interage com a proteína do envelope e protege os vírions imaturos contra a fusão prematura com a membrana do hospedeiro, do modo que a sua liberação implica na maturação da partícula viral (Li et al., 2008). De maneira geral, esta proteína desempenha um papel importante no arranjo e maturação da partícula do DENV (Dwivedi et al., 2017).

A principal e maior proteína estrutural do DENV é a glicoproteína do envelope (E), 53 kDa, composta por 390 resíduos de aminoácidos organizados em quatro domínios: EDI, EDII, EDIII e uma haste proximal à membrana que se conecta com a âncora transmembrana. O terceiro é responsável pela atividade de ligação ao receptor celular, enquanto o segundo contém o peptídeo hidrofóbico crucial para a ligação e fusão do vírus à membrana da célula alvo (Dwivedi et al., 2017). A proteína E se dispõe em forma de dímero na partícula viral matura, e é o principal alvo para anticorpos neutralizantes (Heinz & Stiasny, 2012). Tem sido observado particularmente para DENV-2, mudanças conformacionais sutis desta proteína influenciadas por temperatura e por cátions divalentes, denominadas "respiração". Esta dinâmica estrutural própria do envelope de DENV-2 está correlacionada com a infectividade viral, não assim as morfologias individuais adotadas. Acredita-se que possa ser uma estratégia que permita modular o acesso a certos epítopos e assim, o escape do reconhecimento de anticorpos (Sharma et al., 2019).

#### *1.3.2.2 Proteínas não estruturais*

Dentre as proteínas não estruturais, a NS1 de 46 kDa é uma glicoproteína envolvida no complexo de replicação do RNA. A proteína é sintetizada como um monômero de 352 aa, que após processamento no retículo endoplasmático e na rede trans-Golgi, ou bem se mantem em forma de dímero associada ao complexo de replicação viral ou à membrana plasmática, ou é secretada ao espaço extracelular e ao sangue como uma partícula de lipoproteína hexamérica (Muller et al., 2013). Esta forma secretada interage e inibe componentes do sistema imune mediados por complemento (Akey et al., 2014). Sendo assim, a NS1 extracelular resulta em um alvo de reconhecimento e controle do sistema imunológico humoral, o que por outro lado permitiu que tenha sido empregada como alvo no desenho de ensaios imunoenzimáticos (ELISA) e ensaios imunocromográficos rápidos (Peeling et al., 2010). Em cada monômero se distinguem três domínios diferentes. O primeiro é o pequeno domínio "rolo-β" (aa 1 a 29) envolvido na dimerização O segundo domínio (aa 30 a 180) se projeta do domínio central beta como uma asa, forma que dá o seu próprio nome. Este domínio “asa” contém dois locais de glicosilação (Asn130 e Asn175), e dois subdomínios discretos, um dos quais cria junto com o domínio rolo-β, uma saliência com uma superfície marcadamente hidrofóbica, conservada nos DENV.

Tem sido proposto que é através desta região que NS1 interage com a membrana do RE e outras proteínas transmembranares virais, como NS4A e NS4B (Akey et al., 2014). O terceiro domínio é a característica estrutural predominante de NS1 e trata-se de uma folha beta contínua que se estende ao longo do comprimento do dímero como os degraus de uma escada. Este domínio "escada-β" central é formado pela metade C-terminal de NS1 (aminoácidos 181 a 352), e conjuntamente com o domínio asa, estão expostos ao solvente. Acredita-se que esta região está envolvida na interação com as proteínas estruturais e assim, na montagem dos vírions (Scaturro et al., 2015).

As proteínas NS2A e 2B são duas proteínas de membrana. NS2A, de 22 kDa e 218 resíduos de aa apresenta no seu extremo N-terminal, dois segmentos localizados no lúmen do RE que foram relacionados à cito-patogênese do DENV, enquanto a região C-terminal encontra-se implicada na montagem e liberação do vírion. Esta proteína intervém também na síntese do RNA viral, sendo co-localizada junto ao RNA viral dupla fita (dsRNA, do inglês “double-stranded RNA”) e interagindo com estruturas do extremo 3’ não codificante dentro do complexo de replicação, e possivelmente também com NS3 e NS5. Por outro lado, tem sido demostrado que NS2A atua sinergicamente com NS4B na inibição da resposta celular antiviral (Xie et al., 2014; Gopala Reddy et al., 2018). Pela sua vez, a proteína NS2B de 14 kDa e 130 aa, encontra-se também inserida na membrana do RE, deixando um segmento hidrofílico de 40 resíduos exposto no citosol. O mesmo se associa de modo não covalente com o domínio protease da NS3, atuando como cofator, permitindo o correto enovelamento, a localização e atividade de serinoprotease viral. Foi observado que este complexo NS2B3 participa também na imunomodulação por parte do hospedeiro, inibindo a resposta do interferon (INF) do tipo 1 e estimulando a via apoptótica nas células endoteliais (Gopala Reddy et al., 2018). Por outro lado, acredita-se que NS2B também cumpra um rol no complexo de replicação, uma vez que tem sido observada a sua co-localização com o dsRNA, e também como viroporina, o que sugere que facilitaria o efeito citopático induzido pelo DENV e ao mesmo tempo a montagem e secreção viral (Li et al., 2015).

A proteína NS3 de 70 kDa e 618 resíduos de aa é uma proteína multifuncional com atividade enzimática de serina protease semelhante a quimotripsina no seu domínio N-terminal (resíduos 1-168), e atividades RNA helicase, RNA trifosfatase e NTPase no domínio C-terminal (resíduos 180-618). De este modo, NS3 está envolvida

na clivagem da poliproteína, bem como na replicação do RNA (Rice, 1996; Luo et al., 2008). Ambos os domínios da proteína se encontram ligados por um fragmento de 11-12 aa, altamente flexível. Esta característica tem um profundo efeito na eficácia da replicação do genoma viral (Lou et al., 2010). Por outro lado, além de conformar junto com a proteína NS5, o centro catalítico do complexo de replicação, tem sido demostrado que NS3 é necessária para a produção de partículas virais infectivas, de modo que mutações pontuais podem abolir a formação de partículas infeciosas sem afetar a tradução, o processamento da poliproteína ou a replicação do RNA viral (Gebhard et al., 2016).

As proteínas NS4A e NS4B são, tal como NS2A e B, duas proteínas transmembrana. A NS4A é uma proteína altamente hidrofóbica, de 16 kDa e 150 aa, dos quais, a porção terminal chamada 2K de 23 aa após a clivagem pela protease viral se encarrega de dirigir a NS4B ao lúmen do RE, para posteriormente ser clivada desta última por uma signalase celular. A região N-terminal da NS4A (aa 1-47) se encontra localizada no citoplasma celular, e se trata de uma região anfipática a qual interage com a membrana do RE e está envolvida na indução da curvatura desta membrana, facilitando desta forma a invaginação da mesma, para posterior liberação do vírion imaturo no lúmen do RE. Essa curvatura permite também a montagem do complexo de replicação. Tem sido determinado, que mutações que alteram o mencionado caráter anfipático, abolem a replicação viral *in vitro*. O domínio transmembrana C-terminal (aa 52-119) está envolvido na oligomerização da própria NS4A, que acontece antes da indução da curvatura, e é um processo essencial para a remodelagem da membrana. Pela sua vez, é também através desta região que NS4A interage com NS4B, o que se acredita é a chave para modular a transição desde a formação das bolsas de vesícula para a formação do complexo de replicação viral (Gopala Reddy et al., 2018). A glicoproteína NS4B de 27 kDa e 248 resíduos de aa, também está envolvida na replicação viral por meio da sua interação através do loop citoplasmático (aa 125-162) com NS3 (Zou et al., 2015b). Pela sua vez, o seu extremo C-terminal (aa 217-248) sofre mudanças conformacionais e passa desde o lúmen do RE para o citoplasma, onde interage com NS5 (Gopala Reddy et al., 2018). Tanto NS4A como NS4B interagem com NS1 para modular a replicação viral (Zou et al., 2015). Por outro lado, estas duas proteínas de membrana também cumprem um papel fundamental na regulação da resposta imune inata do hospedeiro, prevendo indiretamente a indução da via do Interferon (INF) (Zou et al., 2015). No entanto, o rol

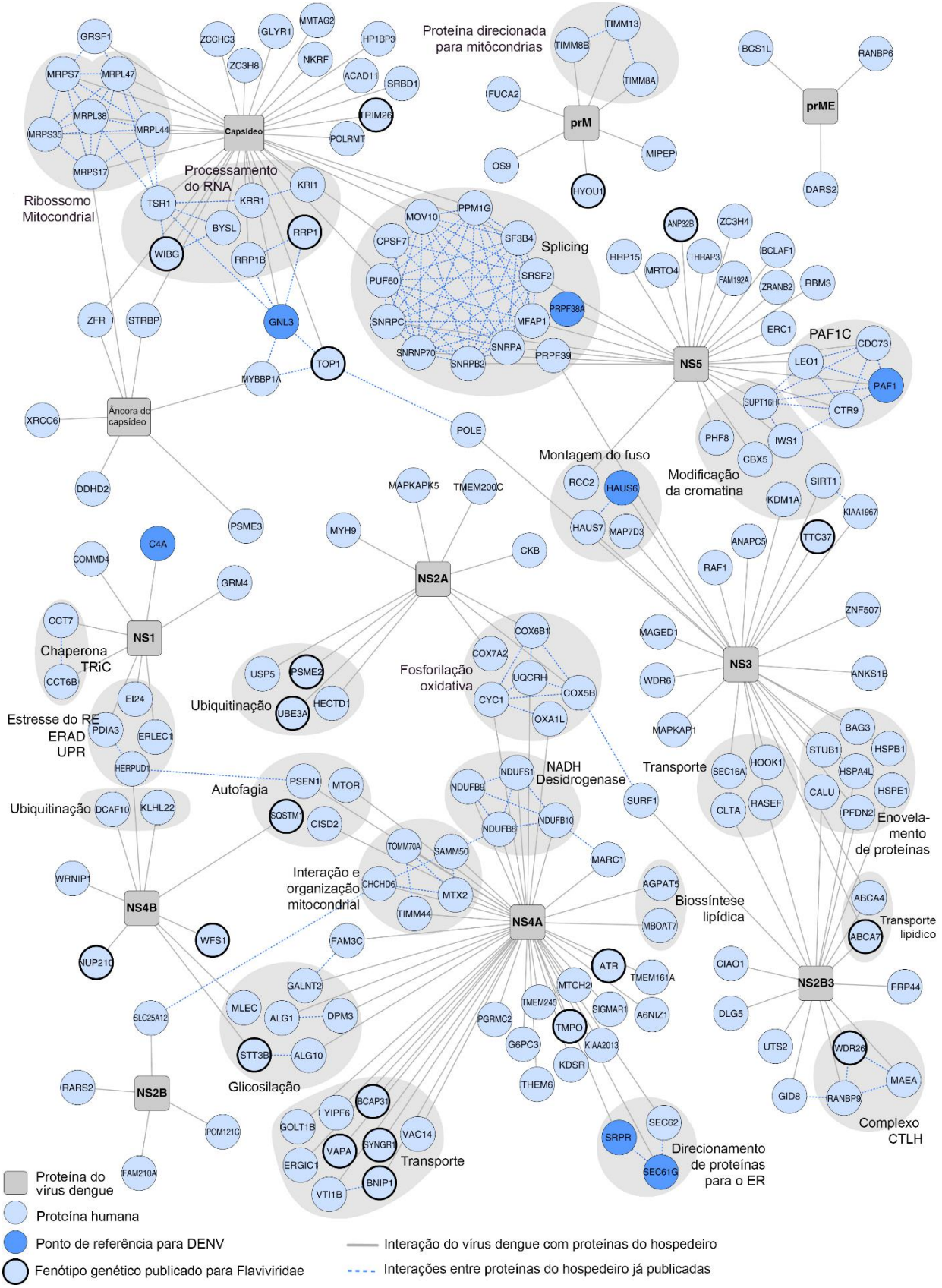
de NS4B é mais amplo, desde que foi demostrado que suprime tanto a resposta de desnaturação de proteínas quanto a formação de grânulos de estresse em resposta à infecção viral (Zmurko et al., 2015). Além disso, atua como supressor na via do RNA de interferência (RNAi), provavelmente por inibir a endoribonuclease DICER, evitando assim, a biogênese de RNAi curtos (Kakumani et al., 2013).

A NS5 é a maior e mais conservada das proteínas do DENV, apresentando 67% de identidade entre os quatro sorotipos. Esta proteína de 900 resíduos (104 kDa) contém dois grandes domínios unidos por meio de um peptídeo flexível (aa 264-272) que permite que os mesmos adotem diferentes conformações em relação ao outro, e assim dá lugar à interação com diferentes proteínas como NS3, proteínas do hospedeiro, ou diretamente o RNA viral (El Sahili & Lescar, 2017). O domínio N-terminal possui atividade de metiltransferase e se encontra localizado nos resíduos 1–263. Atua também como guanililtransferase, atividade fundamental no processo do "capping" que protege o extremo 5' do genoma viral de ser degradado por endonucleases (Yap et al., 2010). O domínio C-terminal, com atividade de RNA polimerase dependente de RNA, está localizado nos resíduos 273–900. Nele se acham também dois sinais de localização nuclear entre os resíduos 320 e 405, as quais são reconhecidas por fatores celulares, entre eles a α/β-importina, permitindo o transporte de NS5 ao núcleo. Se suspeita que o efeito que esta proteína pode causar no núcleo, induzindo a síntese da quimiocina IL-8, possa ter relação com a patogênese do DENV (Yap et al., 2007; Tay et al., 2016).

Resulta importante destacar que, além das próprias interações entre todas elas e as suas funções na replicação viral, a grande maioria das proteínas virais interage também com diversas proteínas do hospedeiro (Figura 1.4), de maneira que sequestram os processos celulares para favorecer a própria replicação e mecanismos patogênicos, regulando assim as atividades celulares, e as respostas antivirais montadas (Shah et al., 2018).

**Figura 1.4** - Rede de Interação entre as proteínas do DENV e as proteínas humanas. Fonte: imagem adaptada de Shah et al., 2018. São mostradas 198 interações em as diferentes proteínas virais (quadrados cinza) e as da célula humana (círculos azuis).

As interações vírus-hospedeiro se denotam com linhas cinza, enquanto às hospedeiro-hospedeiro com linhas azuis tracejadas.



#### *1.3.2.3 Regiões não codificantes*

Tanto a 5'UTR quanto a 3'UTR são regiões do genoma do DENV altamente estruturadas que portam elementos essenciais para a replicação do vírus.

A região 5'UTR contém dois elementos definidos essenciais para a replicação viral. O maior deles (nucleotídeos 3-70), é uma estrutura haste-alça chamada SLA, do inglês "stem-loop", que interage diretamente com NS5, ativando-a e promovendo assim a polimerização da fita negativa do RNA a partir do extremo 3' do genoma viral em estado circular. Esta estrutura com forma de Y é altamente conservada entre os flavivírus. Em seguida, uma região espaçadora poliU (nucleotídeos 71-76) atua também como promotora da replicação viral, porém resulta prescindível para a circularização do genoma viral. A segunda estrutura identificada em 5'UTR é também uma haste-alça chamada SLB, formada pelos nucleotídeos 77-97. Este elemento por si só não resulta essencial para a replicação viral, mas contém uma sequência de 16 nucleotídeos, conhecida como 5'UAR (do inglês "upstream AUG region", fazendo referência ao códon de iniciação da tradução), que é complementar a uma região presente na extremidade 3' do genoma viral (3'UAR) (Alvarez et al., 2008; Lodeiro et al., 2009).

Já na região 3'UTR, de aproximadamente 450 nt de comprimento, se distinguem três domínios diferentes. O domínio I que é altamente variável, contém as estruturas SLI e SLII ("stem-loops" I e II), enquanto o domínio II contém as estruturas duplicadas DB1 e DB2 (do inglês "dumbell-like") que atuam como promotores da replicação viral, além de modular a circularização do genoma viral. Estas quatro estruturas formam pseudo-nós com regiões próximas, de maneira que ganham maior estabilidade (Alvarez et al., 2005). As estruturas SL e DB duplicadas são capazes de paralisar a degradação do genoma viral pela 5' exonuclease XRN1 celular, resultando no acúmulo de RNAs subgenômicos, chamados de sfRNA, que são relevantes na patogênese viral e na evasão da resposta imune (Clarke et al., 2015). O significado biológico de manter duas estruturas quase idênticas na região 3'UTR ainda não está totalmente claro, mas foi evidenciado que resulta crítico para a passagem do vírus entre as duas espécies de hospedeiro, permitindo que o vírus acomode mutações benéficas em um hospedeiro (mosquitos), mas deletérias em outro (humanos), conferindo robustez durante a troca de hospedeiro (Villordo et al., 2015; de Borba et al., 2019). O terceiro domínio, o mais conservado, contém a sequência CS1 envolvida na circularização do genoma viral, e os elementos sHP (do inglês "short hairpin") e 3'

SL, de 14 e 79 nucleotídeos respetivamente. O último interage com proteínas virais e do hospedeiro para modular a síntese e tradução do RNA viral (Gebhard et al., 2011).

Uma breve e resumida descrição da posição de cada gene codificante no genoma e das regiões 5' e 3' UTRs, como também a suas funções principais são apresentadas na Tabela 1.1:

**Tabela 1.1** Localização e função de cada um dos componentes do genoma viral

| Região /Gene | Posição no genoma | Função |
|---|---|---|
| **5'UTR** | 1-96 | Contém elementos essenciais para a ciclização do genoma viral, e para a replicação viral (Alvarez et al., 2008; Lodeiro et al., 2009). |
| **C** | 97-396 | Componente estrutural do nucleocapsídeo. Contém elementos importantes para a circularização e replicação do genoma (Murphy, 1980; Clyde et al., 2008; Friebe et al., 2011). |
| **prM/M** | 397-936 | Arranjo e maturação da partícula viral (Dwivedi et al., 2017) |
| **E** | 937-2421 | Montagem da partícula viral, interação com receptores celulares e fusão de membranas, principal alvo para anticorpos neutralizantes. (Heinz & Stiasny, 2012; Dwivedi et al., 2017) |
| **NS1** | 2422-3477 | Envolvida na montagem da partícula viral, cofator no processo de replicação viral, evasão do sistema imune (Muller et al., 2013; Akey et al., 2014; Scaturro et al., 2015). |
| **NS2A** | 3478-4131 | Participa na replicação viral, promove a montagem e liberação da partícula viral, inibe a resposta antiviral (Xie et al., 2014; Gopala Reddy et al., 2018). |
| **NS2B** | 4132-4521 | Cofator de NS3, inibe a resposta antiviral mediada por INF, atua como viroporina (Li et al., 2015; Gopala Reddy et al., 2018). |
| **NS3** | 4522-6375 | Serinoprotease, RNA helicase, RNA trifosfatase, NTPase (Rice, 1996; Luo et al., 2008) |
| **NS4A** | 6376-6756 | Arranjo da membrana do RE, formação do complexo de replicação, regulação da resposta imune (Zou et al., 2015; Gopala Reddy et al., 2018). |

| | | |
|---|---|---|
| **NS4B** | 6826-7569 | Intervém na replicação viral, regulação da resposta imune (Zou et al., 2015; Zou et al., 2015b; Gopala Reddy et al., 2018). |
| **NS5** | 7570-10269 | Metiltransferase, Guanililtransferase, RNA polimerase RNA dependente (Yap et al., 2007; Yap et al., 2010; El Sahili & Lescar, 2017). |
| **3'UTR** | 10270-10723 | Contém elementos essenciais para a ciclização do genoma viral, replicação viral, e processos de imunomodulação (Gebhard et al., 2011; Clarke et al., 2015; Villordo et al., 2015). |

#### 1.3.2.4 Modelagem molecular

A função biológica de uma proteína é ditada pelo arranjo dos átomos na sua estrutura tridimensional. Este pode ser o arranjo de resíduos catalíticos em um sítio ativo ou como uma proteína interage com outras proteínas para fins estruturais ou regulatórios. Conhecer a estrutura de uma proteína fornece um nível maior de compreensão de como essa proteína funciona. No entanto, resolver estruturas de proteínas apresenta certos desafios dado que se empregam técnicas como a cristalografia de raios-X ou a ressonância magnética nuclear, que requerem um treinamento extremamente especializado, um alto grau de habilidade, e resultam técnicas de um elevado custo (Kihara, 2020).

Por outro lado, os rápidos avanços das técnicas de sequenciamento nas últimas duas décadas têm permitido o conhecimento cada vez maior das sequências codificantes das proteínas, o qual superou, no ano de 2019, em 1700 vezes a disponibilidade de estruturas conhecidas no banco de dados de proteínas “Protein Data Bank” (PDB) (DNASTAR, 2020). Desta forma, tornou-se claro que, com a tecnologia de hoje, era necessário implementar estratégias alternativas para prever a estrutura de uma proteína. Em consequência, diversas ferramentas de bioinformática foram desenvolvidas e são hoje amplamente aplicadas na previsão destas estruturas. As mesmas se classificam dentro das três possíveis estratégias para obter uma estrutura tridimensional terciária de uma proteína: modelagem de homologia, métodos de enovelamento inverso (conhecidos no inglês como “threading”) e métodos “ab initio ou de novo”. A modelagem por homologia é baseada na suposição de que proteínas com sequências semelhantes tendem a ter estruturas semelhantes e no fato experimental de que a estrutura terciária é mais conservada do que a sequência de

aminoácidos. Desta maneira, esta abordagem, que é uma das mais amplamente usadas e que fornece resultados mais acurados, emprega o alinhamento de sequências de aminoácidos para identificar proteínas nos bancos de dados que possuam um alto grau de similaridade e já tenham sido resolvidas experimentalmente. Desta maneira, as mesmas serão empregadas como modelos a partir dos quais será criada a estrutura da proteína sob estudo. Na ausência de estruturas determinadas experimentalmente, são empregadas as outras duas abordagens. Os métodos de enovelamento inverso se aproveitam dos métodos de modelagem comparativa, porém, não requerem uma única proteína relacionada. Em vez disso, faz a comparação com diversas estruturas de proteínas depositadas nos bancos de dados, e combina as diversas possibilidades (por exemplo, vários fragmentos estruturais curtos extraídos de diferentes proteínas conhecidas) com testes estatísticos. Esta abordagem se apoia no fato de que o número de enovelamentos diferentes na natureza é bastante pequeno (aproximadamente 1300). Finalmente, os métodos “de novo ou ab initio” visam prever estruturas terciárias a partir das sequências, combinando a física (acessibilidade prevista ao solvente, contatos internos previstos, etc) com os comportamentos conhecidos de estruturas secundárias de proteínas, sem o uso de estruturas explícitas (Kihara, 2020).

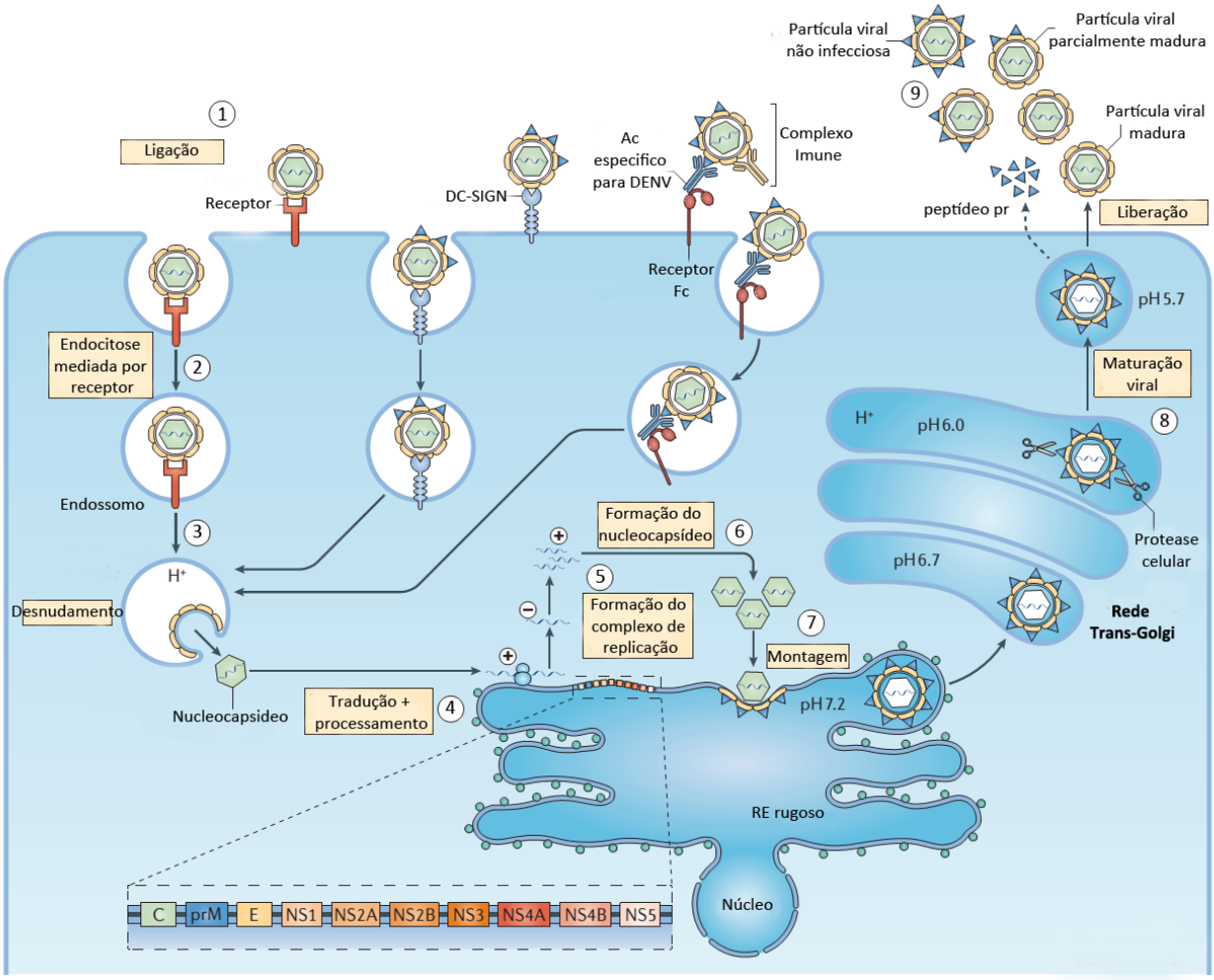
No caso particular do DENV, estruturas resolvidas experimentalmente existem para as proteínas estruturais (C, prM e E) (Ma et al., 2004; Li et al., 2008; Rouvinsky et al., 2015) e três das proteínas não estruturais (NS1, NS3 e NS5) (Luo et al., 2008; Akey et al., 2014; El Sahili et al., 2019). A partir das mesmas é que se conseguiu um entendimento maior e mais detalhado das funções descritas anteriormente. No entanto, para as proteínas não estruturais NS2A-B e NS4A-B apenas se conhecem as suas topologias. As mesmas foram propostas sobre a base de ensaios bioquímicos e/ou ressonância magnética nuclear de pequenos fragmentos, o que foi crucial para obter pedaços de sua estrutura, para em último lugar, montar o quebra-cabeça da topologia mais provável (Xie et al., 2013; Li et al., 2015; Li et al., 2016; Li et al., 2018). Estas quatro proteínas se inserem naturalmente na membrana do retículo endoplasmático (RE), aonde adquirem o seu enovelamento final. Por este motivo, ninguém foi capaz ainda de modelá-las adequadamente em sua totalidade

### *1.3.3 Replicação dos DENV*

Após a transmissão do vírus que ocorre através do repasto sanguíneo pelo vetor, o vírus infecta inicialmente as células de Langerhans e células dendríticas presentes na pele (Wu et al., 2000). Posteriormente o vírus é replicado no citoplasma de células musculares estriadas e/ou lisas, fibroblastos e linfonodos, e após um período de 2 a 7 dias surgem os primeiros sintomas que coincidem com o período de viremia (Kurane & Ennis, 1992).

O processo de interação vírus-célula tem início com a ligação do DENV a receptores presentes na superfície das células do hospedeiro, seguido da endocitose das partículas virais dependente de clatrinas, proteínas celulares envolvidas na formação de vesículas membranares nas células eucariontes (Figura 1.5 passos 1 e 2) (Fragnoud et al., 2012). Após a endocitose, uma mudança conformacional dependente de pH permite o escape do RNA viral desde o endossomo ao citoplasma (Figura 1.5 passo 3). O genoma viral serve como RNA mensageiro, que após ser traduzido pelos ribossomos celulares, dá origem a uma poliproteína que será posteriormente clivada nas diferentes proteínas virais (Figura 1.5 passos 4 e 5). Em seguida, o RNA viral é replicado no retículo endoplasmático perinuclear, intermediado por um RNA de polaridade negativa que serve como molde para a replicação (Figura 1.5 passo 4). O RNA viral associa-se com as proteínas do capsídeo, o qual brota desde a membrana do retículo endoplasmático envolto por uma bicamada lipídica que contém as proteínas virais da membrana (prM/M) e do envelope (E), constituindo assim o envelope viral (Figura 1.5 passos 6 e 7). As partículas virais completas transitam pela via secretora celular e saem finalmente da célula pelo processo de exocitose (Figura 1.5 passos 8 e 9) (Welsch et al., 2009).

**Figura 1.5** Ciclo de replicação do DENV




Fonte: imagem adaptada de Screaton et al., 2015. (+): RNA simples fita polaridade positiva; (-): RNA simples fita polaridade negativa; RE: retículo endoplasmático; Ac: anticorpo; DC-SIGN: proteína da superfície celular específica das células dendríticas.

## 1.4 Diversidade dos DENV

Antigamente, os DENV se classificavam apenas nos quatro sorotipos DENV 1-4 na base das suas relações antigênicas, determinadas mediante ensaios sorológicos de neutralização cruzada (Calisher et al., 1989). Posteriormente, a utilização de análises moleculares e filogenéticas permitiu a classificação do DENV em grupos ou genótipos geneticamente distintos dentro de cada sorotipo, dada a extensiva variabilidade dentro de cada grupo (Holmes & Twiddy, 2003, Vasilakis & Weaver, 2008). Tem sido demonstrado por diversos estudos a existência de cinco genótipos para o DENV-1 (Rico-Hesse, 1990; Weaver & Vasilakis, 2009; Chen & Vasilakis,

2011), seis genótipos para o DENV-2 (Rico-Hesse et al., 1997; Weaver & Vasilakis, 2009; Chen & Vasilakis, 2011), cinco genótipos para DENV-3 (Lanciotti et al., 1994) e quatro genótipos para DENV-4 (Lanciotti et al., 1997; Villabona-Arenas et al., 2011; Chen & Vasilakis, 2011) (Tabela 1.2). Os quatro sorotipos compartilham, em termos de sequências de aminoácidos, identidades que variam entre 60-70% para a proteína do envelope, enquanto que dentro de cada sorotipo, as semelhanças atingem 90% (Weaver & Vasilakis, 2009; Pierson & Diamond, 2013). Inicialmente, as análises de genotipagem e filogenéticas, baseavam-se no sequenciamento do gene do envelope, o mais amplamente utilizado, uma vez que neste gene estrutural existe uma grande probabilidade de achar alterações de nucleotídeos e de aminoácidos, pois está associado com a imunogenicidade do vírus e a resposta de anticorpos do hospedeiro. No entanto, o rápido desenvolvimento e o maior acesso às técnicas de sequenciamento massivo atualmente disponíveis, tornaram cada vez mais comum o desenvolvimento destas análises com sequências virais completas.

**Tabela 1.2** Genótipos descritos para cada sorotipo de DENV

| Sorotipo | Genótipo | Distribuição geográfica |
|---|---|---|
| DENV-1 | I | Sudeste Asiático, China, Leste da África |
| | II | Tailândia (1950-1960) |
| | III | Malásia (cepas selváticas) |
| | IV | Ilhas do Oeste do Pacífico e Austrália |
| | V | Américas, Oeste da África Africano, Ásia |
| DENV-2 | I (Americano) | América Latina, Caribe (1950-1960) |
| | II (Cosmopolita) | Austrália, Leste e Oeste Africano, Ilhas dos oceanos Pacífico e Índico Subcontinente Indiano e Oriente Médio |
| | III (Asiático/Americano) | Tailândia, Vietnã, Américas (últimos 25 anos) Subcontinente Indiano e Ilhas do Pacífico |
| | IV (Asiático I) | Malásia e Tailândia |
| | V (Asiático II) | Vietnã, China, Taiwan, Sri Lanka e Filipinas |

| | | |
|---|---|---|
| | VI (Selvático) | Oeste Africano e Sudeste Asiático (cepas isoladas em humanos, mosquitos silvestres ou macacos sentinelas) |
| DENV-3 | I | Indonésia, Malásia, Filipinas e Sul da Ilhas do Pacífico |
| | II | Tailândia, Vietnã e Bangladesh |
| | III | Sri Lanka, Índia, África, Samoa, Tailândia (1962) |
| | IV | Porto Rico, Américas Latina e Central, Taiti (1965) |
| | V | Filipinas (1956), Japão (1973), China (1980) América do Sul (2002-2004) |
| DENV-4 | I | Tailândia, Filipinas, Sri Lanka e Japão |
| | II | Indonésia, Malásia, Taiti, Caribe e América (provenientes do Sudeste Asiático) |
| | III | Tailândia (1990) |
| | IV | Malásia (cepas selváticas) |

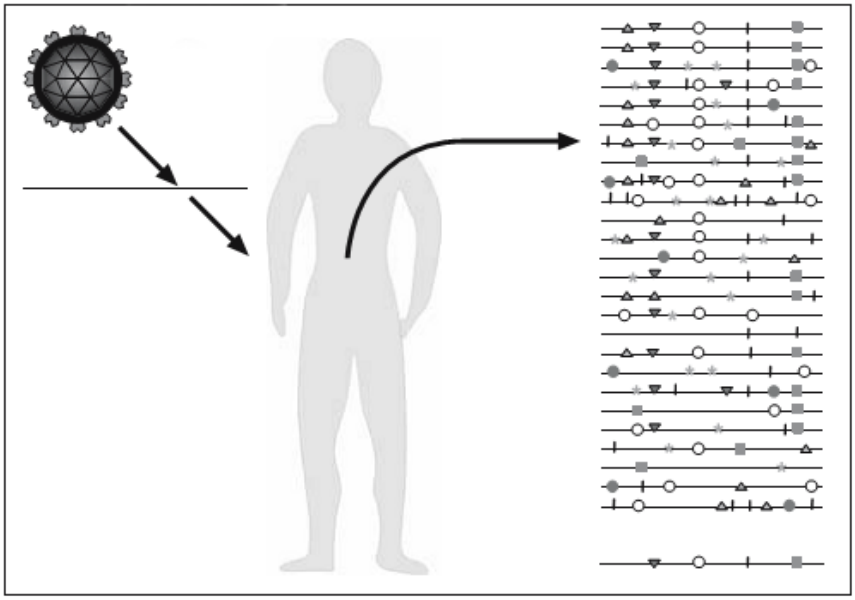
No Brasil, até 2013, eram detectados somente seis genótipos: genótipo V de DENV-1 (dos Santos et al., 2011; Drumond et al., 2012), genótipo III ou Asiático/Americano de DENV-2 (Oliveira et al. 2010; Drumond et al., 2013), genótipos II e III de DENV-3 (Araújo et al., 2009) e os genótipos I e II de DENV-4 (Nunes et al., 2012). Dentro dos diversos genótipos, tem sido descoberta a existência de linhagens, as quais apresentam relações geográficas e temporais diferentes, tanto nas Américas como na Ásia (Myat Thu et al., 2005; Carrillo-Valenzo et al., 2010; Mendez et al., 2010; Duong et al., 2013). Essa diversidade intra-genótipo para DENV é, no entanto, limitada, sendo modulada por dois processos principais, a resposta imune do hospedeiro e os gargalos na transmissão, tanto no hospedeiro vertebrado quanto no invertebrado (Grenfell et al., 2004). Análises de epidemiologia molecular do sorotipo DENV-2, alvo deste estudo, tem demostrado que desde 1990, o genótipo circulante no Brasil é o III, antigamente chamado de Asiático/Americano, o qual foi associado com aumentos na gravidade da doença. Na re-emergência deste sorotipo no ano 2007, com a concomitante mudança do padrão clinico-epidemiológico da dengue no Brasil, estava envolvida uma variante viral pertencente ao mesmo genótipo, mas agrupada em um grupo monofilético diferente às variantes até então circulantes. Desta

maneira foram identificadas filogenética e epidemiológicamente três linhagens dentro daquele genótipo: a linhagem I, das variantes virais que circulavam no Brasil desde 1990, a linhagem II que circulou apenas pelo Nordeste do país durante os anos 2000-2005, e a linhagem III, do período da re-emergência (Oliveira et al., 2010; Drumond et al., 2013). No entanto, a análise da região codificante do gene do envelope de variantes representativas das diferentes manifestações clínicas, não identificou mutações no genoma viral que estivessem associadas a severidade da doença, com a exceção da substituição do aminoácido Asparagina (N) na posição 390, o qual representa um marcador genético de virulência (Pryor et al., 2001).

### *1.4.1 Diversidade intra-hospedeiro de DENV*

A RNA polimerase dependente de RNA do DENV é uma enzima de baixa fidelidade e, portanto, propensa a introduzir variabilidade genética na população viral durante cada ciclo de replicação de RNA. Consequentemente, novas variantes virais são geradas continuamente dentro de um único hospedeiro, moldando o que é definido como "diversidade intra-hospedeiro" (Holmes, 2009). A distribuição dinâmica de mutantes gerada, também conhecida como espectro ou exame de mutantes (Figura 1.6), resulta também de uma sequência de processos de recombinação e tempos de geração curtos na dinâmica evolutiva dos vírus. Essa robustez mutacional permite então em muitas circunstancias o escape à pressão do sistema imune, e assim, à persistência delas no hospedeiro, e tem se relacionado também com o desenvolvimento de rápida resistência às vacinas e às drogas antivirais (Holmes, 2009; Lauring & Andino, 2010; Poh et al., 2013).

**Figura 1.6** Representação esquemática da diversidade viral intra-hospedeiro



Fonte: imagem adaptada de Domingo et al., 2006. Após a infecção no hospedeiro susceptível, o vírus, cujo genoma se representa do lado esquerdo da figura com uma linha reta, se replica nas células do hospedeiro e adquire diversas variações gênicas, se apresentando como um espectro de mutantes conformado por subpopulações virais. As linhas horizontais do lado direito da figura representam aos genomas virais de cada subpopulação, e os símbolos, às mutações próprias de cada uma delas. A última destas linhas representa a sequência consenso da população viral geral.

Foi demonstrado já para muitos modelos virais como a diversidade genética intra-hospedeiro resulta vantajosa para os vírus de RNA, facilitando a sua adaptação a diferentes ambientes e hospedeiros (Sullivan et al., 2007; Lee et al., 2008; Fitzsimmons et al., 2018; Vignuzzi et al., 2019), e como pode contribuir significativamente na patogênese viral, permitindo a modulação da expressão de características fenotípicas distintas (Vignuzzi et al., 2006; Zanini et al., 2015; Moratorio et al., 2017). Por outro lado, foi descrito que os espectros de mutantes podem incluir genomas de memória que reflitam a história evolutiva de uma linhagem viral, do mesmo jeito em que poderia participar na extinção da mesma mediante mutagêneses letais (Ruiz-Jarabo et al., 2003; Briones et al., 2006).

Particularmente para o DENV, diversos estudos têm demonstrado a presença de distintas subpopulações coexistindo dentro de um mesmo hospedeiro (Lin et al., 2004; Descloux et al., 2009; Puiprom et al., 2011; Kurosu 2011; Chao 2012;

Parameswaran et al., 2012; Thai et al., 2012; Kurosu et al., 2013; Romano et al., 2013; Lequime et al., 2015; Rodriguez-Roche et al., 2016; Parameswaran et al; 2017). Alguns deles inclusive têm analisado a diversidade dessas subpopulações em casos correspondentes a infecções primárias e secundárias. Neste contexto, Kurosu e colaboradores acharam uma maior diversidade nos casos das infecções primárias em comparação com os das infecções secundárias, sugerindo que a maior homogeneidade nas infecções secundárias poderia dever-se à presença de anticorpos neutralizantes, o que provocaria a seleção da variante viral de maior *fitness*[1], e possivelmente maior virulência. Como consequência, quadros de dengue mais graves poderiam se desenvolver (Kurosu, 2011; Kurosu et al., 2013). Rodriguez-Roche e colaboradores pelo contrário, observaram uma diversidade maior nos casos das infecções secundárias (Rodriguez-Roche et al., 2016). Outro estudo em Taiwan, que analisou clusters familiares onde ocorriam subsequentemente casos de dengue e dengue grave (casos na sua maioria primários), propôs que a transmissão mecânica pelo mosquito vetor, de subpopulações menores, porém mais virulentas e com maior *fitness*, possa estar favorecida e causando casos graves entre membros da família, posteriores aos casos de dengue clássico que ocorriam primeiro nessa mesma família (Chao, 2012).

Nos últimos anos, ferramentas como o sequenciamento de nova geração (NGS) permitiram uma abrangência total dos genomas virais e em elevada profundidade, dando lugar à identificação e quantificação dessas subpopulações virais geradas no hospedeiro, ou seja, no curso de uma única infecção, com elevada precisão e acurácia, sendo uma das ferramentas mais sensíveis no campo da genômica. Inúmeros estudos em diferentes modelos virais têm obtido resultados na análise da biodiversidade de subpopulações dentro do ecossistema do hospedeiro com a aplicação destas tecnologias de sequenciamento profundo (Matranga et al., 2014; Yin et al., 2012; Romano et al., 2013; Grubaugh et al., 2015; Lequime et al., 2015). O uso de este tipo de técnica de alto rendimento corroborou com provas adicionais a todo o anteriormente exposto para DENV, demonstrando que variações de *fitness* e adaptabilidade viral ocorriam sem mudanças na sequência viral consenso

[1] *Fitness*: parâmetro importante da genética adaptado por virologistas para quantificar a capacidade de replicação de um vírus e produzir uma progênie infecciosa. Geralmente é determinado em experimentos de competição em cultura de células ou *in vivo*, junto com um de vírus de referência (Domingo et al., 2012).

(Parameswaran et al., 2012; Rodriguez-Roche et al., 2016; Parameswaran et al., 2017).

## 1.5 Transmissão

Fatores relativos ao ambiente e intrínsecos do mosquito, do DENV e do hospedeiro influenciam a dinâmica da transmissão dessa doença e o seu controle (Valle et al., 2015).

O DENV é mantido na natureza por um ciclo de transmissão que envolve aos hospedeiros vertebrados e aos mosquitos hematófagos do gênero *Aedes* (Gubler, 2002). Na África e na Ásia os DENV se mantêm circulando em ciclos urbanos, silvestres e rurais, enquanto nos outros continentes a circulação é basicamente urbana, envolvendo mosquitos com hábitos domésticos, como é o caso do *Aedes aegypti.* Em algum momento no passado, humanos ou mesmo primatas não humanos adquiriram a infecção por meio da exposição à picada de mosquitos silvestres infectados pelo DENV ao frequentar o ambiente selvagem. Estes hospedeiros, ao circularem virêmicos no ambiente modificado, permitiram a propagação do vírus por mosquitos domésticos (*Ae. aegypti* no caso da África e *Ae. albopictus* na Ásia), tornando esse ciclo totalmente independente de reservatórios silvestres. A partir do estabelecimento desse ciclo estritamente doméstico e peridomiciliar em portos marítimos, o vírus se espalhou pelo mundo conjuntamente com o seu vetor *Ae. aegypti* (Vasilakis et al., 2011).

A infecção do mosquito vetor fêmea pelo DENV se inicia a partir da ingestão de partículas virais infecciosas presentes no sangue de um humano virêmico. Após o repasto sanguíneo, é necessário um período de incubação que varia entre 8 e 12 dias para que o vírus se replique no estômago do mosquito e invada as suas glândulas salivares. Estas partículas virais agora infectantes serão inoculadas em um novo hospedeiro durante o próximo repasto sanguíneo, determinando um novo ciclo (Salazar et al., 2007).

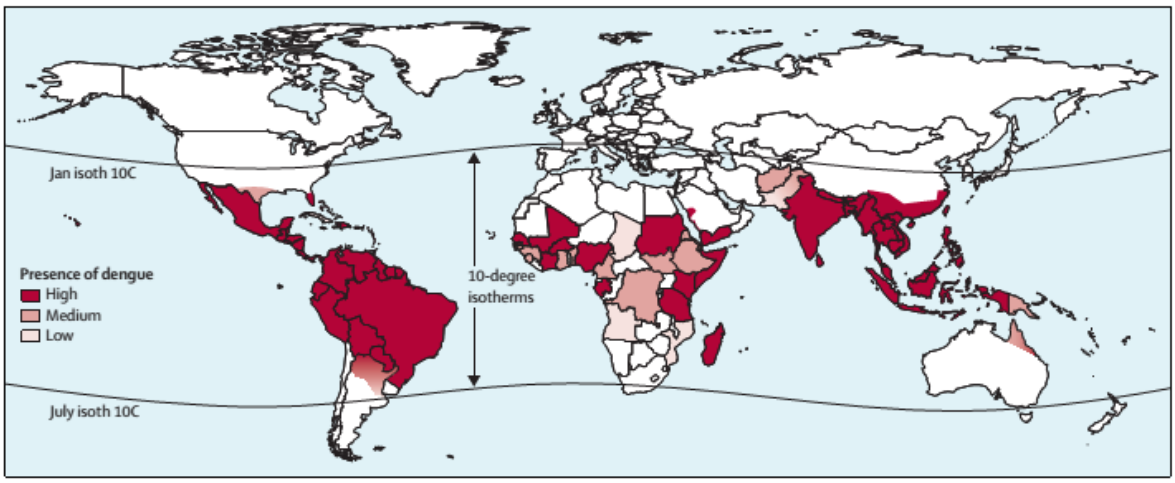
Existe, ainda, uma transmissão do vírus da fêmea do mosquito infectada para a sua progênie, por via transovariana ou vertical (Anderson & Rico-Hesse, 2006), assim como também uma transmissão venérea, a partir de mosquitos machos que transmitem para as fêmeas durante a cópula (revisado em Kramer & Ebel, 2003).

## 1.6 Epidemiologia da doença

### *1.6.1 No mundo*

Essa arbovirose considerada inicialmente benigna e sem grandes repercussões, começou a se apresentar já na década de 1950 com epidemias de febre hemorrágica no Sudeste Asiático. Naquele momento, eram só 9 os países envolvidos, e quase 1000 os casos de febre da dengue e febre hemorrágica da dengue reportados à Organização Mundial (OMS) (Gubler, 1997). Hoje, a doença já é endêmica em mais de 100 países nas regiões da OMS da África, Américas, Mediterrâneo Oriental, Sudeste Asiático e Pacífico Ocidental, sendo as regiões da América, Sudeste Asiático e Pacífico Ocidental as mais seriamente afetadas (Figura 1.7). Nos últimos 60 anos, a sua incidência tem aumentado pelo menos 30 vezes com uma crescente expansão geográfica para novos países, incluindo a Europa, e desde áreas urbanas a rurais (Bhatt et al., 2013; WHO, 2020). Na última década, o número de casos notificados à OMS aumentou de 2,4 milhões em 2010 a 4,2 milhões em 2019, ano com o maior número de casos de dengue já relatados globalmente. Esse aumento alarmante no número de casos é parcialmente explicado por uma mudança nas práticas nacionais para registrar e relatar a dengue aos Ministérios da Saúde e à OMS. Porém, também representa o reconhecimento dos governos do que esta carga realmente significa, e, portanto, a necessidade real de notificar os casos. Portanto, embora a carga global total da doença seja incerta, a OMS estima que sejam cerca de 50-100 milhões de infecções, com 500.000 casos de febre hemorrágica por dengue no mundo a cada ano. Porém, uma estimativa obtida por modelagem indicou uma notória subnotificação de casos de dengue com manifestações clínicas, estimando que o número real de casos possa atingir quase os 400 milhões de infecções por ano ocorrendo na América, Ásia, África e Oceania (revisado em WHO, 2020).

**Figura 1.7** Distribuição da dengue no mundo




Fonte: Guzman & Harris, 2015. Representada numa escala de cor rosa, desde o mais claro ao mais escuro, a prevalência da dengue no mundo, de menor a maior respectivamente.

Nas Américas, o vírus dengue começou a recircular no início dos anos 1960, após um período de silêncio epidemiológico, decorrente possivelmente da eliminação do mosquito vetor em vários países do continente (Pinheiro e Corber, 1997). Em 1963, o sorotipo DENV-3 foi isolado na Jamaica de onde começou a se disseminar para as ilhas do Caribe, Venezuela e Colômbia. Nos anos 1968 a 1970, os sorotipos DENV-2 e 3 provocaram epidemias em países da América Central e do Sul, e continuaram circulando pela região durante toda a década de 70, sendo que no final desse período e começo da década de 80, os sorotipos DENV-1 e 4 também foram introduzidos. Desde então, o cenário da dengue foi se agravando, apresentando-se com epidemias consecutivas, atingindo principalmente grandes centros urbanos, e com uma marcada tendência ao aumento da gravidade dos casos, aumentando também a proporção de casos de febre hemorrágica por dengue (San Martin et al., 2010).

### *1.6.2 No Brasil*

Embora os primeiros relatos de uma doença semelhante à dengue no Brasil datem de 1846, com surtos ocorridos simultaneamente nos estados do Rio de Janeiro, Bahia, Pernambuco e em localidades do norte do país (Mariano, 1917), somente a

partir de 1986, a dengue se tornou um problema de Saúde Pública nacional, com a identificação do primeiro caso do DENV-1 no município de Nova Iguaçu, RJ, (Schatzmayr et al., 1986), alcançando um elevado número de notificações em vários estados do Brasil (Miagostovich et al., 1993; Nogueira et al., 1999). Em 1990, um novo sorotipo, o DENV-2 foi isolado na cidade de Niterói (Nogueira et al., 1990). A co-circulação de dois sorotipos e a natureza do genótipo de origem asiática de DENV-2 que foi introduzido nas Américas e posteriormente no Brasil, resultou no agravamento do quadro clínico e a notificação dos primeiros casos de dengue hemorrágico, síndrome de choque por dengue e óbitos no país. Em dezembro de 2000 mais um sorotipo, o DENV-3, foi detectado no município de Nova Iguaçu (Nogueira et al., 2001), sendo responsável até aquele momento pela maior e mais grave epidemia de dengue já descrita no país e no continente americano, não apenas pelo elevado número de notificações (794.200 casos), assim como pela ocorrência de casos graves e fatais (Araújo et al., 2009). Esse agravamento da expressão clínica da doença, se intensificou ainda mais com a re-emergência do sorotipo DENV-2 no ano 2007, ocasionando a grave epidemia do ano de 2008 que acabou afetando todo o país. O estado do Rio de Janeiro, porém, foi o mais acometido, com um total de 198.269 de casos notificados (SVS/MS, 2009). Foi também característico desse período a mudança da faixa etária acometida, sendo as crianças as mais envolvidas (Cavalcanti et al., 2011). Em 2010, o DENV-4 reemergiu em Roraima e a partir daí, ocorreu a disseminação desse sorotipo para o resto do país, e foi o responsável por grande parte dos casos de dengue do ano 2012 (Temporão et al., 2011; SVS/MS, 2012; Brasil, 2014). A circulação deste sorotipo começou a diminuir nos anos posteriores, cedendo o predomínio novamente ao sorotipo DENV-1 (SVS/MS, 2014; SVS/MS, 2015). Nos anos seguintes, esses dois sorotipos continuaram circulando, porém, desde o final de 2014, diferentes arbovírus como o vírus chikungunya (CHIKV), o vírus Zika (ZIKV) e o vírus da febre amarela (YFV), se juntaram alternando sua circulação (SVS/MS, 2017).

Após 2010, entretanto, o DENV-2 manteve uma circulação basal e baixa em nível nacional, sempre abaixo dos sorotipos DENV-1 e DENV-4. Menos de 4% dos casos notificados de dengue pertenciam ao sorotipo DENV-2, e principalmente nas regiões norte e nordeste do país (SVS/MS 2012; SVS/MS, 2017). Esse cenário perdurou até 2016, mas no final de 2017, mesmo sendo o ano com o menor número de casos de dengue registrados nacionalmente, a região Centro-Oeste do país passou a apresentar um predomínio crescente do DENV-2 (SVS/MS, 2018). Ao longo de

2018, manteve a sua difusão a um ritmo reduzido, obtendo, em média, valores semelhantes aos de 2017 (SVS/MS, 2019). Porém, ao longo de 2019, os casos de DENV-2 alcançaram taxas de notificação 282% superiores às do ano anterior, confirmando-se a sua circulação também na região sudeste do país (SVS/MS, 2019b).

## 1.7 Manifestações clínicas e patogênese da doença

O homem é o único hospedeiro capaz de desenvolver a doença, cuja apresentação clínica pode variar desde uma infecção assintomática, até a dengue com ou sem sinais de alarme, ou dengue grave, forma que se bem é rara, pode atingir a letalidade (Figura 1.8). Aproximadamente 90% dos casos, apresentam-se de forma autolimitada, com uma duração máxima de uma semana (WHO, 2009). A febre, acompanhada pelas dores generalizadas e o possível envolvimento gastrointestinal e aparição do exantema maculopalular, define à dengue clássica. Esta etapa, em termos gerais, costuma ser a única fase da doença, e está associada ao período de viremia. Durante esta etapa, não é possível saber se o paciente vai permanecer com sinais e sintomas da dengue clássica, autolimitando e evoluindo para a cura espontânea, ou se o quadro irá se agravar causando uma dengue grave, com choque e até mesmo hemorragias maciças (manifestações decorrentes da perda de líquidos para o espaço extravascular, pelo aumento da permeabilidade vascular). Essa possível etapa crítica, é marcada geralmente pela queda da febre (Martinez, 2008). No entanto, só uma minoria desenvolve o quadro grave, que pode ser fatal. Existem também formas de apresentação classificadas como atípicas (neurológicas, hepáticas, cardíacas, entre outras), menos frequentes que o dengue clássico ou dengue grave, as quais têm sido relatadas em países do Sudeste Asiático e das Américas, inclusive no Brasil (Gulati & Maheshwari, 2007; Valle et al., 2015). Porém, pouco se conhece sobre a incidência destas formas.

**Figura 1.8** Classificação clínica da dengue segundo a OMS


Fonte: Imagem adaptada da Organização Mundial da Saúde (WHO, 2009).

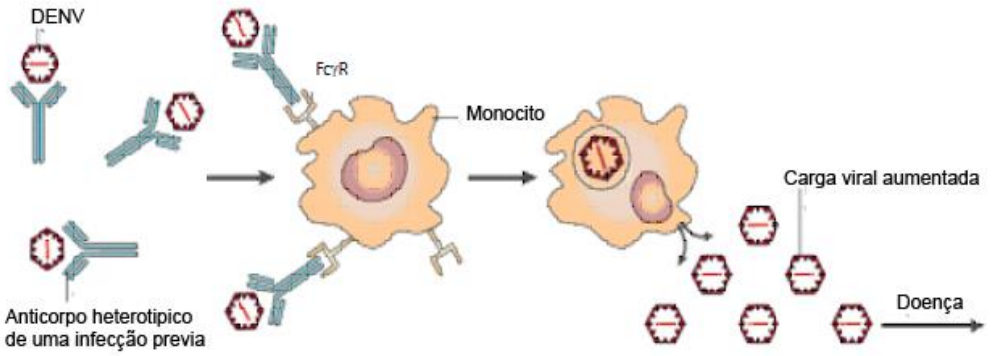
### *1.7.1 Patogênese*

A base fisiopatológica da dengue é multifatorial. Diversas teorias são propostas para explicar o alto grau de variação das manifestações clínicas causadas pelos DENV, no entanto duas são as mais aceitas: a teoria das infecções sequenciais e a da virulência da cepa infectante.

A teoria da infecção sequencial ou da facilitação dependente de anticorpos (do inglês: “ADE - antibody dependent enhancement”), propõe que a resposta imune de um indivíduo sensibilizado é amplificada numa segunda infecção, decorrente da existência prévia de anticorpos heterotípicos e considera que há um aumento da replicação viral em macrófagos via estes anticorpos (acs) heterólogos. Em infecções secundárias com um vírus heterólogo ao da infecção anterior, os anticorpos reagem de forma cruzada com este novo sorotipo, mas não são capazes de neutralizá-los. Esses complexos, ao serem reconhecidos e internalizados por fagócitos mononucleares, resultariam na infecção celular e replicação viral. Essas células infectadas liberam na corrente sanguínea mediadores vasoativos, aumentando a permeabilidade vascular, ativação do sistema complemento e da tromboplastina

tissular, desencadeando os mecanismos responsáveis pelas manifestações clínicas das formas hemorrágicas (Hastead, 1988) (Figura 1.9).

**Figura 1.9** Modelo da teoria das infecções sequenciais por DENV




Fonte: imagem adaptada de Whitehead et al., 2007. A infecção facilitada dependente de anticorpos ocorre quando os anticorpos presentes no corpo, preexistentes a partir de uma infecção primária por DENV, se ligam a uma partícula de DENV durante uma infecção subsequente, com um sorotipo de dengue diferente. Os anticorpos da infecção primária não podem neutralizar o vírus. Em vez disso, o complexo anticorpo-vírus liga-se a receptores chamados receptores Fcγ (FcγR) em monócitos circulantes. Os anticorpos ajudam ao vírus a infectar os monócitos de forma mais eficiente. O resultado é um aumento na replicação global do vírus e um maior risco de dengue grave.

No entanto, cabe destacar que estudos recentes têm determinado que o desenvolvimento do ADE é dependente do título de acs heterólogos, existindo uma janela determinada dentro da qual o risco ante uma segunda infecção por um sorotipo diferente, resulta maior. Quando o título de acs era menor ao limite inferior desta janela, o efeito resultava insignificante, enquanto quando ocorria a situação inversa e o título superava o limite superior da janela, os acs conseguiam limitar a infeção, mesmo tratando-se de acs de tipo heterólogos (Katzelnick et al., 2017; Sajle et al., 2017).

A virulência da cepa infectante, sugere que a gravidade da doença se deve, também, às variações genéticas e antigênicas das diferentes cepas de vírus. Uma explicação admitida, é que a evolução genética do vírus dentro de cada sorotipo possa dar origem a cepas epidêmicas ou mais virulentas (Rosen, 1977; Rico-Hesse, 1990).

Apesar do conjunto complexo de fatores que contribuem para a epidemiologia da doença, diversos estudos sugerem que as estruturas virais específicas podem contribuir para o aumento da replicação em células alvo humanas e aumentar a transmissão pelo mosquito vetor (Rico-Hesse, 2003). Esta teoria poderia ser a responsável de explicar a gravidade clínica nas infeções primárias. Sendo assim, nosso estudo tomará como base, a teoria da virulência da cepa viral, considerando que a evolução do DENV resulte da seleção de cepas com maior virulência que terão impacto direto nos seres humanos.

É importante destacar que embora não sejam o foco deste estudo, diversos fatores genéticos ou previas comorbidades do hospedeiro como asma, diabetes e anemia falciforme também foram apontadas como responsáveis pelo desenvolvimento de quadros de maior gravidade. Variações genéticas nos genes codificantes do receptor de vitamina D, receptor Fcγ IIA, fator de necrose tumoral (TNF) α, interleucina 10, entre outros, foram associados a gravidade da doença (Sierra et al., 2007; Perez et al., 2010). Por outro lado, uma redução da gravidade da dengue em indivíduos de etnias negras foi observada em comparação com indivíduos brancos (Sierra et al., 2007b). Finalmente, um aumento na taxa de admissão hospitalar e letalidade por dengue grave durante infecção secundária foi relatada em crianças quando comparada com adultos; o que se atribui principalmente às diferenças na permeabilidade microvascular entre as diferentes faixas etárias (Gamble et al., 2000; Guzman et al., 2002).

Acredita-se então que o desfecho clinico da infecção pelo DENV depende do equilíbrio entre os antecedentes genéticos e imunológicos do hospedeiro e os fatores virais.

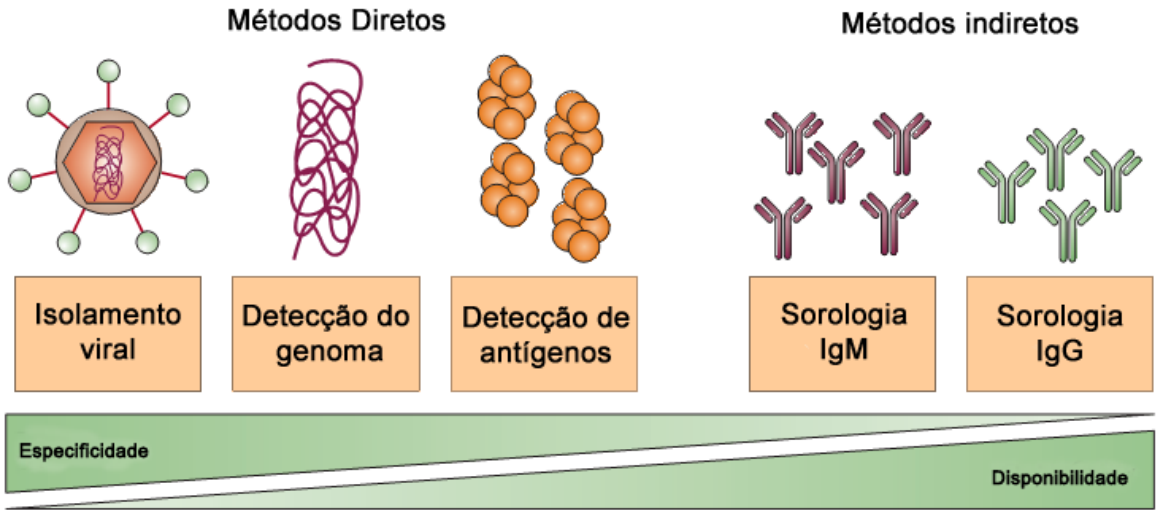
## 1.8 Diagnóstico laboratorial das infecções por DENV

Os testes de diagnóstico laboratorial desempenham um papel crucial na assistência ao paciente, confirmando ou descartando o caso por uma infecção suspeita (Peeling et al., 2010). Nas infecções por DENV, o diagnóstico laboratorial de casos suspeitos é importante para a vigilância da doença, o monitoramento dos sorotipos circulantes do vírus e para o diagnóstico diferencial de outras doenças que causam sinais e sintomas clínicos semelhantes à dengue (Nogueira & dos Santos, 2015).

O diagnóstico laboratorial das infecções por DENV pode ser realizado por métodos diretos que visam isolar ou identificar o vírus, ou os seus componentes (antígenos como por exemplo NS1, e o ácido nucleico viral) ou por métodos indiretos, os quais consistem na detecção de anticorpos específicos de tipo IgM e IgG decorrentes da infecção. O período da doença em que o paciente se encontra é fundamental para a apropriada escolha do método diagnóstico a utilizar e para a correta interpretação dos resultados. A viremia é detectável por aproximadamente 4-5 dias após o início dos sintomas, e correlaciona-se estreitamente com a duração da febre. Nas infecções primárias, os anticorpos de tipo IgG surgem lentamente, com baixos títulos após 8-10 dias desde o começo dos sintomas, enquanto os de tipo IgM são detectados tipicamente após os primeiros 5 dias de iniciados os sintomas e perduram na circulação sanguínea por 2-3 meses. Nas infecções secundárias, no entanto, os anticorpos de tipo IgG surgem rapidamente, e em altos títulos, logo depois do começo da febre, enquanto os do tipo IgM podem ser indetectáveis em alguns casos (WHO, 2009).

Os métodos diretos, que são certamente mais específicos que os indiretos, nem sempre são os mais disponíveis no diagnóstico de rotina das infecções por DENV (Figura 1.10).

**Figura 1.10** Métodos empregados no diagnóstico das infecções por DENV




Fonte: imagem adaptada de Peeling et al., 2010. Os métodos diagnósticos diretos, como o isolamento do vírus, a detecção do seu genoma e a detecção de antígenos virais são formas mais específicas de diagnosticar a infecção por DENV do que os

métodos indiretos, que detectam anticorpos de tipo IgM e IgG anti DENV. Porém, no diagnóstico de rotina, os testes indiretos são as opções mais práticas disponíveis.

O soro é a amostra por eleição, embora plasma, sangue total e fragmentos de tecidos também sejam úteis. A linhagem celular C6/36 derivada de *Ae. albopictus* é o sistema de isolamento viral de escolha para o diagnóstico, porém, a inoculação intratoráxica de mosquitos do gênero *Toxorhynchites* é a alternativa para isolamento de maior sensibilidade. Ensaios de transcrição reversa seguida da reação em cadeia da polimerase (RT-PCR) ou ensaios de imunofluorescência com anticorpos monoclonais sorotipo-específicos são empregados para a identificação dos sorotipos, embora estes últimos tenham caído em desuso devido à maior disponibilidade e facilidade de operação das técnicas de biologia molecular. Por sua vez, ensaios de RT-PCR ou RT-PCR em tempo real são os métodos de escolha para a detecção do genoma viral. A detecção do antígeno viral NS1, secretado pelas células infectadas, oferece a possibilidade também do diagnóstico inicial, pois pode ser detectada até 9 dias após o início da febre, e em amostras de tecidos. Utilizam-se testes rápidos comerciais e ensaios imunoenzimáticos (ELISA). Finalmente, a detecção de anticorpos é o teste mais amplamente utilizado na vigilância laboratorial. Existem diferentes formatos de ELISA que podem ser empregados na detecção dos mesmos (Guzman & Harris, 2015).

## 1.9 Prevenção e controle

O controle da doença tem se tornado cada vez mais problemático. Programas baseados no controle vetorial e programas comunitários que visavam manter o ambiente livre de criadouros trabalharam de maneira constante e ativa, porém, falharam até hoje em erradicar o mosquito vetor. Novas abordagens para o controle vetorial foram desenvolvidas nos últimos dez anos apresentando progressos interessantes: i) a adaptação endosimbiótica da bactéria *Wolbachia* de *Drosophila* no *Ae. aegypti* mostrou efeitos de encurtamento da vida do mosquito e de bloqueio da transmissão direta do DENV (Hoffmann et al., 2011); ii) avanços com *Ae. aegypti* geneticamente modificados carregando um gene dominante letal e a liberação desses mosquitos machos na natureza também se mostrou como uma medida de controle biológico efetiva na redução de índices entomológicos (Harris et al., 2011); iii) novos

óleos essenciais com atividade larvicida e biopesticidas derivados de outros microrganismos estão sendo desenvolvidos (Veerakumar et al., 2013; Dias & Moraes, 2014); iv) uso de armadilhas disseminadoras de inseticida, a partir das quais as próprias fêmeas transferem partículas do inseticida aos criadouros, impedindo-se logo o desenvolvimento do mosquito juvenil (Abad-Franch et al., 2017) .

Por outro lado, em consequência das dificuldades encontradas na implementação de programas de controle do mosquito vetor, junto com o crescente número de casos de dengue no mundo, o desenvolvimento de uma vacina tetra-valente que estimulasse uma resposta imune balanceada e eficiente para os 4 sorotipos virais tornou-se uma grande necessidade.

No entanto, o desenvolvimento de uma vacina contra a dengue enfrenta dois grandes desafios. Em primeiro lugar, embora os anticorpos contra DENV mostrem efeitos protetores contra uma infecção homotípica ou heterotípica, o título de anticorpos pré-existentes ante uma infecção secundária heterotípica pode levar ao desenvolvimento de um quadro grave de dengue pelo efeito ADE (Katzlenick et al., 2017; Sajle et al., 2017). No entanto, a resposta imune e a patogênese da dengue grave não são ainda totalmente compreendidas, o que acaba dificultando o desenvolvimento da vacina. Em segundo lugar, não existe um modelo animal convenientemente acessível, barato e sensível, capaz de simular as respostas imunológicas em humanos após a infecção. Uma vez que os camundongos são naturalmente resistentes à infecção por DENV, apenas modelos quiméricos camundongo-humano, camundongos imunodeficientes sensíveis à infecção por DENV, ou vias de infecção não fisiológicas foram estabelecidos para serem usados como modelos animais; todos eles apresentando suas limitações (Yauch e Shresta, 2008). Primatas não humanos (PNHs) são modelos animais de alto potencial porque produzem uma resposta imune à infecção por DENV semelhante à dos humanos, mas geralmente são usados após testes em camundongos por causa do custo (Sariol e White, 2014).

Diversas estratégias têm sido empregadas na tentativa de desenvolver uma vacina eficaz contra o DENV. Como por exemplo:

a- Vacina Viva Atenuada: modelos de vírus vivo, porém, cuja virulência é reduzida a níveis considerados seguros para a aplicação clínica. Apresenta as vantagens de fornecer um conjunto de antígenos protetores e proteção imunológica de longo prazo (Whitehead et al., 2007). A vacina desenvolvida por Sanofi Pasteur,

Dengvaxia®, é a única que até hoje cumpriu estudos de eficácia em fase III (Hadinegoro et al., 2015), foi certificada pela Organização Mundial da Saúde em 2016 e foi licenciada em 21 países endêmicos para dengue, como Filipinas, Malasia, México, Brasil e Paraguai. Esta vacina tetravalente, viva e atenuada, consiste em uma estrutura recombinante do cDNA infeccioso da cepa vacinal do vírus da Febre Amarela (17D) cujos genes prM e envelope foram substituídos pelos correspondentes aos 4 sorotipos do DENV (Guy et al., 2011; Sanofi Pasteur, 2016). No entanto, após a conclusão dos estudos em fase III e a implementação da vacina nos países licenciados, um número inesperado de hospitalizações por infecção clínica por dengue foi observado significativamente entre os participantes vacinados, principalmente nos menores de 9 anos de idade. Análises retrospectivas de diferentes coortes envolvidas nos ensaios de fase III determinaram que aqueles destinatários da vacina que não haviam sido previamente expostos ao DENV ou com imunidade anti-dengue limitada manifestaram um risco maior de dengue grave após a vacinação com Dengvaxia. Títulos muito baixos de anticorpos desenvolvidos após a vacinação e/ou diferenças na eficiência da vacina de acordo com os quatro sorotipos infectantes teriam sido os causantes da manifestação de ADE (Katzelnick et al., 2017; Sajle et al., 2017). Sendo assim, em abril de 2018, a OMS publicou as últimas recomendações do Grupo Consultivo Estratégico de Especialistas em Imunização (SAGE) sobre o uso da vacina Dengvaxia em áreas endêmicas da dengue. Até a presente data, o SAGE recomenda a implantação da vacina Dengvaxia® somente em regiões endêmicas em que a soroprevalência da dengue seja superior a 70% na população-alvo, excluindo indivíduos com menos de 9 anos de idade. O limite de idade para a vacinação dependeria do nível de transmissão da dengue na área em questão, mas geralmente é de 45 anos. Cabe destacar que, em vista ao anteriormente exposto, se faz necessário poder contar com um ensaio sorológico de alta especificidade e sensibilidade, o qual, conforme exposto anteriormente, representa ainda hoje um grande desafio justamente nos países endémicos aonde há circulação paralela de outros Flavivírus. Por outro lado, evidências obtidas em populações humanas e modelos de camundongos sugerem um papel de proteção exercido pelas células T CD8+, com a maioria dos epítopos localizados nos genes não estruturais (Yauch et al., 2009). Desta forma, a vacina quimérica de Sanofi

Pasteur carece assim, de resposta das células T às proteínas não estruturais do vírus dengue.

Outros dois modelos de vacina viva e atenuada encontram-se sob estudos clínicos de fase III. A DENVax, desenvolvido pela Takeda Vaccine Incorporated, consiste também numa vacina tetravalente quimérica, criada a partir do esqueleto genómico do vírus DENV-2 cepa 16681, um vírus atenuado por passagem seriada em cultura primária de células renais de cão (DENV-2 PDK53), a partir do qual os genes prM e E foram substituídos pelos respetivos dos outros três sorotipos (Brewoo et al., 2012). Embora esta vacina tenha se mostrado capaz de ativar respostas humorais e de células T específicas para as proteínas não estruturais de DENV-2 (Sharma et al., 2019), resultando isto em uma vantagem com respeito à Dengvaxia, a eficácia para DENV-4 observada nos estudos em fase III ainda não resulta clara (Biswal et al., 2019). Por outro lado, a vacina LAVDelta30 (TV003/TV005), criada pelo National Institute of Allergy and Infectious Diseases dos Estados Unidos (US NIAID) e em co-desenvolvimento com o Instituto Butantan, é também uma versão tetravalente cuja atenuação foi causada a partir da deleção de 30 nucleotídeos na região 3' não codificante. Esta última apresentou resultados mais promissores na resposta imune ao DENV-2, quando comparada à Denvaxia, e induziu soroconversão para os quatro sorotipos em 92% dos indivíduos vacinados (Stephen et al., 2003; Kirkpatrick et al., 2015), o que a converte numa possível futura candidata no controle profilático da doença.

b- Vacinas inativadas: dentre os diferentes modelos que têm sido desenvolvidos, a vacina tetravalente TDENV PIV, formulada a partir de vírus inativados com formalina, e administrada junto com adjuvantes tem gerado títulos robustos e persistentes de anticorpos neutralizantes contra os quatro sorotipos após duas doses administradas em macacos *Rhesus* (Fernandez et al., 2015). Paralelamente, resultados preliminares de um ensaio clínico em fase I tem demostrado que quando administrada em humanos, seguida por uma dose de reforço de uma vacina tetravalente atenuada, se induzem títulos mais elevados de anticorpos neutralizantes e uma taxa mais alta de soroconversões tetravalentes em comparação com a administração na ordem contrária (Lin et al., 2020). As vantagens deste tipo de vacinas são o perfil de segurança aceitável que apresentam em amplas faixas etárias e em hospedeiros imunocomprometidos, e que resultam adequadas para coadministração com outras vacinas.

c- Subunidades recombinantes: trata-se de proteínas antigênicas expressadas em células procariotas ou eucariotas para estimular respostas imunes protetivas a longo prazo. Comparadas com as vacinas atenuadas, estas têm maiores chances de gerar respostas imunes balanceadas contra os quatro sorotipos e menores de induzir ADE, no entanto, quando se utiliza um sistema de expressão procarioto, podem existir falhas no enovelamento das proteínas alvo, assim como também contaminação com endotoxinas do sistema de expressão (Deng et al., 2020). Diversos modelos têm sido criados a partir do domínio III da proteína do envelope, combinados com adjuvantes (Chen et al., 2013), ou utilizando outras proteínas de fusão como uma lipoproteína (Chiang et al., 2016), P64K de *N. meningitidis* (Lazo et al., 2011) ou LTB de *S. cerevisiae* (Bal et al., 2018), entre outras. Embora todos eles tenham tido sucesso como imunógenos em camundongos, o modelo mais prometedor é V180, desenvolvido pela Hawaii Biotech Inc. e, em seguida, adquirida pela Merk & Co., Incorporated. Esta vacina é composta por uma proteína do envelope correspondente a cada sorotipo, truncada nos resíduos 393-395 (DENV-E80) e expressada em células S2 de *Drosophila*. A vacinação de camundongos e macacos *Rhesus* com baixas doses da mesma induziu um alto nível de imunidade protetora (Govindarajan et al., 2015).

d- Vetores virais: Diferentes modelos virais têm sido modificados por engenharia genética para atuarem como vetores na apresentação de antígenos de DENV. Modelos com vetores adenovirais têm apresentado muitas vantagens, como fácil manipulação de genes, fácil detecção de defeitos de replicação e alto nível de expressão de proteínas, e têm se demostrado eficientes imunógenos quando empregados na vacinação de camundongos (Smita et al., 2003; Khanam et al., 2007). Particularmente, o cAdVaxD (1–2) e cAdVaxD (3–4), duas vacinas vetoriais de complexo divalente de adenovírus (cAd) que expressam prM e E dos DENVs, apresentaram resultados positivos na indução de anticorpos contra os quatro sorotipos e proteção imunológica de células T em macacos *Rhesus* (Raviprakash et al., 2008). Por outro lado, vetores de alfavírus têm se mostrado de alto potencial no desenho de vacinas contra DENV. Partículas de replicon do vírus (VRP) da encefalite equina venezuelana expressando as proteínas M e E do DENV-1 têm induzido a produção de anticorpos protetores em macacos *Cynomolgus* e *Rhesus* (Chen et al., 2007; White et al., 2013). Assim mesmo, a vacina tetravalente VRP expressando o domínio III da proteína do envelope (E85-VRP) induziu uma

resposta imunológica balanceada e proteção contra DENV1–4 em macacos com 2 doses administradas com 6 semanas de intervalo (White et al., 2013). Acredita-se que as vacinas contra DENV de vetores virais sejam a maneira mais segura, e talvez eficaz, de induzir imunidade celular (Deng et al., 2020).

e- Vacinas de DNA: este último tipo de vacinas consiste num plasmídeo contendo um ou mais genes codificantes de antígenos específicos de DENV, que ao serem inoculados in vivo, se expressam nas células produzindo os antígenos que atuarão estimulando a resposta imune do indivíduo vacinado. São modelos estáveis, fáceis de produzir em massa e de baixo custo, porém, também de baixa imunogenicidade. Vacinas de DNA expressando prM e um fragmento do envelope têm sido testadas em camundongos com resultados satisfatórios, enquanto macacos *Aotus nancymae* resultaram parcial ou totalmente protegidos contra DENV-1 quando administradas duas doses da vacina D1ME100, portadora dos genes codificantes de prM e envelope completos (Kochel et al., 2000; Raviprakash et al., 2000). Esta última foi pela sua vez, testada em humanos num ensaio clínico de fase I e mostrou-se segura e bem tolerada, porém, a imunogenicidade resultou baixa; apenas 41,6% dos sujeitos que receberam uma alta dose produziu anticorpos neutralizantes, e nenhuma resposta foi detectada no grupo de baixa dose (Beckett et al., 2011). Portanto, uma formulação tetravalente deste tipo de vacina desenvolvida pela US Naval Medical Research Center dos Estados Unidos, foi testada em combinação com um adjuvante de maneira de melhorar sua imunogenicidade. Os resultados dos ensaios de fase I demostraram que esta vacina resultou segura e bem tolerada. Enquanto as respostas anti-dengue de células T ocorreram na maioria dos indivíduos do estudo, as respostas de anticorpos neutralizantes foram fracas, o que leva a desenhar possíveis métodos alternativos de administração, bem como abordagens de dose-reforço, que possam resultar em uma resposta imune humoral mais robusta e duradoura (Danko et al, 2018).

Embora tenham sido desenvolvidos múltiplos modelos de vacinas contra DENV, e já exista uma aprovada, apenas algumas outras candidatas conseguiram avançar nos ensaios clínicos (Tabela 1.3). Existe ainda uma necessidade crucial de entender melhor a dinâmica viral nas infecções naturais para poder atingir um desenho de vacina que permita uma resposta tetravalente balanceada, além de cumprir com os requisitos básicos de segurança e eficácia.

**Tabela 1.3** Vacinas candidatas contra a dengue em uso ou em ensaios clínicos.

| Tipo de vacina | Nome | Estratégia | Fase nos ensaios clínicos |
|---|---|---|---|
| **Viva atenuada** | Dengvaxia | Substituição dos genes prM/E no genoma do YFV-17D pelos de DENV 1-4 | Licenciada |
| | TV003/TV005 | Atenuação por deleção de 30 nt na região 3' UTR de DENV-1, 3 e 4, e a quimera DENV-2/4 | Fase III |
| | DENVax | Substituição no genoma do vírus atenuado DENV-2 PDK-53 dos genes prM e E pelos respetivos dos outros três sorotipos | Fase III |
| **Vírus inativado** | PIV | Virus purificado e inativado com formalina + adjuvantes | Fase I |
| **Subunidade** | V180 | Proteína E recombinante truncada no aa 394-396 | Fase I |
| **DNA** | D1ME100 | Plasmídeo recombinante que codifica para prM/E de DENV-1 | Fase I |
| | TVDV | Plasmídeo recombinante que codifica para prM/E dos 4 sorotipos | Fase I |

Fonte: adaptado de Deng et al., 2020. YFV: vírus da febre amarela; PDK: células de cultura primária de rim de cão.

# 2 JUSTIFICATIVA

Atualmente, além de ser um país hiper endêmico para DENV com a co-circulação dos quatro sorotipos de DENV e padrões epidemiológicos cada vez mais graves, o Brasil tem enfrentado também grandes surtos de vírus da zika e de febre amarela (SVS 2017a-b), para os quais a reatividade cruzada com o DENV, já foi comprovada por diferentes autores (Bradina et al., 2017, Keasey et al., 2017). Exposições prévias a outros Flavivírus ou a imunização com a vacina Dengvaxia (já licenciada e disponível na rede privada) podem atuar como fatores de seleção permitindo o surgimento de novas cepas virais. Já foi proposto que, devido às pressões de seleção convergentes, novos “hotspots” onde gerar diversidade no genoma viral podem surgir dentro de cada hospedeiro (Parameswaran et al., 2017). Sim e colaboradores demonstraram que as populações virais são capazes de restaurar rapidamente sua diversidade após um evento de restrição, criando predominantemente um repertório de variantes muito diferente, que provavelmente se origina de mutações aleatórias (Sim et al., 2015). Por outro lado, a elevada variabilidade genética evidenciada no DENV-2 também tem sido apontada como causa do insucesso em testes com vacinas (Sabchareon et al., 2012; Whitehead et al., 2016).

Particularmente, o sorotipo DENV-2 foi o responsável por uma das epidemias de maior impacto na história do Brasil, evidenciando-se em 2008 um marcado aumento na gravidade da doença, com elevada taxa de mortalidade e uma mudança concomitante na faixa etária afetada (SVS/MS, 2009). Todo isto leva a necessidade de novos estudos que permitam compreender melhor a evolução do DENV-2 e o seu impacto na doença.

Diversos estudos têm demonstrado uma forte associação entre a composição genética das populações virais intra-hospedeiro com o *fitness* e patogenia viral, como foi descrito para o Poliovírus (Vignuzzi et al., 2006; Fitzsimmons et al., 2018), HIV (Lee et al., 2008; Zanini et al., 2015), HCV (Sullivan et al., 2007), Vírus Coxsackie B3 e Influenza A (Moratorio et al., 2017), assim como muitos outros revisados em Vignuzzi et al., 2019, permitindo a modulação da expressão de características fenotípicas distintas por diferentes subpopulações virais. Por outro lado, foi demonstrado também para vírus de RNA que apenas uma ou algumas substituições de aminoácidos dentro de uma única proteína são suficientes para modificar uma determinada característica

biológica do vírus (Pfeiffer et al., 2003; Tsetsarkin et al., 2007). Pela sua vez, são inúmeros os estudos de mutagêneses dirigida nas diferentes proteínas ou estruturas secundárias do genoma viral de DENV que exibiram claras correlações entre substituições de nucleotídeos e alterações nas funções das mesmas, com impacto na replicação viral (Hsieh et al., 2014; Xie et al., 2014; Lee et al., 2015; Scaturro et al., 2015; Gebhard et al., 2016).

Considerando o exposto, a diversidade intra-hospedeiro assume um lugar de alta relevância no estudo da evolução das populações de DENV durante o curso da infecção humana e sua relação com a gravidade da doença. Identificar variantes virais com a capacidade de impactar no desfecho clínico dos pacientes, pode servir como ponto de partida para o desenvolvimento ou melhoria de testes diagnósticos que permitam a identificação precoce de possíveis quadros de maior gravidade, assim como também aproveitar variantes limitadas por defeitos de replicação ou custos de adaptação para a seleção inteligente de novos candidatos e alvos para o desenho de vacinas e/ou drogas.

Com o objetivo de compreender melhor a dinâmica evolutiva do DENV, e o seu impacto na gravidade da doença, este estudo analisou a relação entre a diversidade genética intra-hospedeiro do DENV-2 com a gravidade do quadro clínico apresentado por pacientes dos estados do Rio de Janeiro, São Paulo e Minas Gerais, durante o período de 2007 a 2019. Para tal, descrevemos as características genéticas virais e sua relação com a gravidade da doença ou com o tipo de infecção (primária ou secundária). Desta forma, a informação gerada neste estudo retrospectivo e analítico, é de suma relevância, porque gera informações importantes que podem auxiliar no controle de uma doença com elevado impacto social e econômico.

# 3 OBJETIVOS

## 3.1 Objetivo Geral

Analisar a diversidade genética intra-hospedeiro de DENV-2 em amostras de pacientes com dengue e dengue grave, identificando potenciais variantes virais de impacto no desenvolvimento da infecção e na patogênese severa da doença.

## 3.2 Objetivos Específicos

1) Determinar o genótipo e linhagem de DENV-2 envolvido em cada amostra, para realizar uma correta seleção das amostras que formarão parte do estudo;

2) Analisar e comparar a diversidade dos espectros de mutantes virais identificados nas amostras de DENV-2 selecionadas;

3) Descrever a relação entre o tipo de infecção (primária/secundária), quadro clínico (dengue, dengue com sinais de alarme e dengue grave) e a diversidade genética achada nas amostras de DENV-2 selecionadas;

4) Determinar se existem pontos quentes mutacionais (do inglês “hotspots”) em alguma região do genoma viral em particular de acordo com a classificação do quadro clínico;

5) Construir modelos em três dimensões das diferentes proteínas virais, e determinar qual o impacto das substituições de nucleotídeo único achadas consistentemente nos espectros de mutantes das diferentes amostras sobre a estrutura destas, inferindo possíveis mudanças na estrutura e função das mesmas;

6) Determinar qual o impacto das substituições de nucleotídeo único achadas sobre as estruturas secundárias de RNA existentes nas regiões não codificantes do genoma viral, inferindo possíveis impactos estruturais e no *fitness* viral.

# 4 METODOLOGIA E RESULTADOS

Sobre a base dos objetivos planteados no presente estudo, esta secção será dividida em três eixos para facilitar a compreensão de como eles se articularam conforme o desenvolvimento do projeto: 1) DENV-2 no período 2018-2019; 2) Definição da amostragem e análise da diversidade intra-hospedeiro; 3) Análise de mutações de relevância. Os resultados obtidos para cada um deles serão apresentados em forma de artigos publicados (eixos 1 e 2) ou submetido à publicação (eixo 3) em revistas indexadas.

Cabe destacar que este estudo se encontra registrado no Sistema Nacional de Informação sobre Ética em Pesquisa (SISNEP) e dentro dos objetivos aprovados pelo Comitê de Ética em Pesquisa (Certificado de Apresentação para Apreciação Ética (CAAE) número 90249219.6.1001.5248, Parecer 2.998.362; Cadastro no SISGEN número A3BDF12) da Fundação Oswaldo Cruz, Ministério da Saúde (Anexo I).

## 4.1 DENV-2 no período 2018-2019

A dissertação que precede a este trabalho visou analisar a diversidade intra-hospedeiro em duas linhagens diferentes do genótipo Asiático/Americano de DENV-2, atualmente chamado de genótipo III, para em última instância poder associá-la com a gravidade do quadro clínico desenvolvido pelos indivíduos infectados. Amostras de 27 pacientes do Estado do Rio de Janeiro foram estudadas, sendo apenas 6 da linhagem BR1, envolvida na introdução do genótipo no Brasil em 1990, e 21 da linhagem BR3, envolvida no grande surto causado entre os anos 2008-2010. Conforme foi concluído, analisar a diversidade intra-hospedeiro em duas linhagens filogeneticamente distantes e com antecedentes de alterações fenotípicas também distintas, resultava em um fator desconexo na hora de discernir acerca da relação entre a diversidade genética viral observada nos pacientes e a gravidade do desfecho clinico (Torres, 2016). Por outro lado, resultou evidente a necessidade de ampliar a amostragem desde que apenas os 21 casos correspondentes á linhagem BR3 resultavam escassos para poder tirar conclusões acuradas das tendências observadas.

Paralelamente a isto, no final de 2017 começou-se a detectar-se uma baixa circulação do sorotipo DENV-2 na região centro-oeste do Brasil, após de quase uma década de silencio epidemiológico. Durante o ano 2018 a circulação se manteve baixa, porém em 2019 houve um recrudescimento dos casos, atingindo as restantes regiões do país (SVS, 2019; SVS, 2019b). Surgiu desta forma a incógnita sobre a origem desta cepa viral e a sua relação filogenética com a linhagem BR3 que foi introduzida por último no Brasil, assim como também acerca da possibilidade desta cepa ser incluída no estudo de diversidade intra-hospedeiro de DENV-2. Sendo assim, se desenvolveu a análise do artigo exposto em seguida, e se colaborou com outros trabalhos posteriores de similares abordagens (de Goes et al., 2020; Ribeiro Adelino et al., 2021).

**4.1 Artigo 1** - Re-introduction of dengue virus serotype 2 in the state of Rio de Janeiro after almost a decade of epidemiological silence

**Relação do manuscrito com os objetivos:** Os resultados apresentados neste manuscrito são referentes ao seguinte objetivo:

1) Determinar o genótipo e linhagem de DENV-2 envolvido em cada amostra, para realizar uma correta seleção das amostras que formarão parte do estudo;

**Situação do manuscrito:** Artigo publicado na revista *Plos One*.

**Fator de Impacto da Revista:** 2,74.

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PLOS ONE

RESEARCH ARTICLE

# Re-introduction of dengue virus serotype 2 in the state of Rio de Janeiro after almost a decade of epidemiological silence

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## Abstract

The Asian/American genotype of dengue virus serotype 2 (DENV-2) has been introduced in Brazil through the state of Rio de Janeiro around 1990, and since then it has been spreading and evolving, leading to several waves of dengue epidemics throughout the country that cause a major public health problem. Of particular interest has been the epidemic of 2008, whose highest impact was evidenced in the state of Rio de Janeiro, with a higher number of severe cases and mortality rate, compared to previous outbreaks. Interestingly, no circulation of DENV-2 was witnessed in this region during the preceding 9-year period. By early 2010, phylogenetic analysis of the 2008 epidemic strain revealed that the outbreak was caused by a new viral lineage of the Asian/American genotype, which was pointed as responsible for the outbreak severity as well. The same scenario is repeating in 2019 in this state; however, only a few cases have been detected yet. To provide information that helps to the understanding of DENV-2 dynamics in the state of Rio de Janeiro, and thereafter contribute to public health control and prevention actions, we employed phylogenetic studies combined with temporal and dynamics geographical features to determine the origin of the current viral strain. To this effect, we analyzed a region of 1626 nucleotides entailing the Envelope/NS1 viral genes. Our study reveals that the current strain belongs to the same lineage that caused the 2008 outbreak, however, it is phylogenetically distant from any Brazilian strain identified so far. Indeed, it seemed to be originated in Puerto Rico around 2002 and has been introduced into the state in late 2018. Taking into account that no DENV-2 case was reported over the last decade in the state (representing a whole susceptible children generation), and the fact that a new viral strain may be causing current dengue infections, these results will be influential in strengthening dengue surveillance and disease control, mitigating the potential epidemiological consequences of virus spread.

design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

## Introduction

Arboviral infections have been re-emerging in Brazil over the last decades, threatening the country and causing a constant burden for public health [1, 2]. Human travelers and extensive migrations have facilitated the spread of arbovirus potentially threatening for human health through the globe [3, 4]. As a tourist magnet, the state of Rio de Janeiro, in southeast Brazil, has been facing large arbovirus epidemics year by year [5].

Dengue virus (DENV) is an arbovirus that belongs to the *Flaviviridae* family, *Flavivirus* genus, and is transmitted to humans by mosquitoes from genera *Aedes*. Four genetically related but antigenically distinct DENV serotypes have been described: DENV-1, DENV-2, DENV-3, and DENV-4. Besides Brazil, DENV is widely distributed across tropical and subtropical areas of the world [6].

In 1986 the serotype DENV-1 was identified in the state of Rio de Janeiro, and since then, dengue fever became a public health burden for Brazil [7]. By early 1990, the Asian/American (As/Am) genotype of serotype DENV-2 was isolated from patients from the metropolitan region of the state [8]. From then on, its spreading gave rise to the epidemic of the years 1990–1991, leading to the advent of the first severe forms of the disease [9]. These two serotypes were also responsible for the epidemic waves of 1995–1996 and 1998, from which the virus spread rapidly to other districts [10]. Already in 2000, the serotype DENV-3 was firstly isolated from a patient in the state of Rio de Janeiro as well. The introduction of this new serotype was associated with an increase in the number of cases during the summer of 2001–2002, causing the largest epidemic so far in the state [11]. This serotype circulated predominantly in the state until 2007, when serotype DENV-2 reemerged, causing a massive and severe epidemic during 2008 that affected the entire country. The state of Rio de Janeiro, however, was the most affected, with more than 235,000 reported cases, over 13,000 hospital admissions and 263 deaths [12], which represented increased disease severity and a higher rate of mortality, compared to previous outbreaks [13]. This epidemic caused staggering human and economic costs, with more than US$1 billion being spent at the national level on dengue prevention and control [14]. Drumond et al. demonstrated that the virus causing the 2008 epidemic belonged indeed, to the same genotype that was introduced around 1990, but to a different lineage [15]. In fact, this new lineage presented genetic differences potentially associated with a more pathogenic clinical outcome of the disease [16]. Henceforth, in 2011, the serotype DENV-4 was identified in the municipality of Niterói, state of Rio de Janeiro, and spread the following year to other districts in the state, accounting for most cases of dengue in 2012 and 2013 [17–19]. The circulation of this serotype decreased after that, giving way again to serotype DENV-1 [20]. During the following years, these two serotypes kept on circulating in the state, however, since late 2014, different arboviruses such as chikungunya virus (CHIKV), Zika virus (ZIKV), and yellow fever virus (YFV), joined alternating their circulation [19]. DENV-2 cases remained undetectable in the state since 2010, until 2017, when one new case was reported in the capital of the state [21]. Consequently, the statal health department claimed to intensify surveillance, even though DENV detection during 2017 was low, and the focus was mostly on surveillance of yellow fever cases and epizootics, and immunization actions as well [22]. However, it was only in 2018, when the Information System for Notifiable Diseases (SINAN) and the Laboratory Sample Management System (GAL) registered three new different DENV-2 cases in the state of Rio de Janeiro [23].

After 2009 however, DENV-2 maintained a basal and low circulation at a national level, always below serotypes DENV-1 and DENV-4. Less than 4% of the notified dengue cases belonged to serotype DENV-2 mainly in the north and northeast regions of the country [18, 19]. This scenario abided until 2016, but in 2017, even being the year with the lowest number

of registered dengue cases nationally, the central-west region of the country started to show a growing predominance of DENV-2 [24]. During 2018, it kept on spreading at a low rate, obtaining on average, similar figures as the ones from 2017 [25]. However, throughout this current year, DENV-2 cases reached notification rates 282% superior as the preceding year, and its circulation has been already confirmed also in the southeast region of the country, including the state of Rio de Janeiro [26].

Considering that after almost a decade of epidemiological silence of DENV-2 in the state, a whole new generation is naïve to this serotype infection and concerned about a potential new outbreak of great magnitude in the state, we sought to briefly analyze the virus phylogeny and its origin, to contribute to public health policies by providing additional information that may help to strengthen monitoring, control and surveillance measures of this viral strain spreading.

## Materials and methods

### Ethical statement

The samples analyzed in this study belong to the collection from the Laboratory of Flavivirus, IOC/FIOCRUZ, Rio de Janeiro, Brazil, and were obtained through the passive surveillance system from an ongoing Project approved by resolution number CAAE 90249218.6.1001.5248 from the Oswaldo Cruz Foundation Ethical Committee in Research (CEP 2.998.362), Ministry of Health-Brazil. Samples were chosen anonymously, based on the laboratorial results.

### Study samples

Sera samples were obtained from ten patients with suspected dengue fever from the districts of Vassouras, Volta Redonda, Nova Iguaçu e Mangaratiba (west of the state of Rio de Janeiro), between January and March of the current year. They were sent to the Central Laboratory of Rio de Janeiro LACEN/RJ for diagnostic confirmation through RT-PCR under Lanciotti´s protocol [27]. DENV-2 strain 40247 (Produced by Bio-Manguinhos/FIOCRUZ, RJ) [28] was employed as a positive control, while a normal human serum sample collected from a healthy donor was used as a negative one.

### DENV-2 genetic sequencing

Viral strain sequencing was performed by the Regional Reference Flavivirus Laboratory at Instituto Oswaldo Cruz/FIOCRUZ in Rio de Janeiro. Briefly, viral RNA was extracted from 140 ul of serum samples using the QIAamp Viral RNA Mini Kit (Qiagen, CA-EUA), followed by a RT-PCR to amplify a small portion of 1639 nucleotides entailing the Envelope (ENV)/NS1 region (1467–3106 according to AF489932 reference sequence) of the viral genome, employing primers pair 3A-4B, described elsewhere [29]. For this purpose we used the QIAGEN OneStep RT-PCR Kit (Qiagen, CA-EUA) according to manufacturer's instructions, and. Thermocycling conditions consisted of a single step of 50˚C for 30 minutes for reverse transcription and 95˚C for 15 minutes for reverse transcriptase inactivation, followed by 40 cycles of denaturation at 94˚C (30 seconds), annealing at 63˚C (60 seconds), extension at 72˚C (2 minutes) and a final extension at 72˚C (10 minutes). PCR products were confirmed by 1.5% agarose gel electrophoresis stained with SYBR Safe DNA gel stain (Invitrogen) and visualized under blue light. Subsequently, PCR specific bands were sliced from the gel and purified with the Qiagen Gel Extraction Kit (Qiagen, CA-EUA), following manufacturer's instructions. Quantification of DNA amplicons was carried on with Qubit® fluorometer (Life Technologies) and finally sequenced by Sanger-based technique employing fluorescent dideoxynucleotides at the DNA sequencing Platform at Fiocruz Foundation, Rio de Janeiro.

### Phylogenetic analyses

The obtained sequences (GenBank accession numbers MK972823 and MK972824) were manually edited and aligned with BioEdit v7.2.5.0 software using different DENV-2 reference sequences representative for the different genotypes and available at GenBank (accession numbers can be consulted in S1 Table). Alignment of the final 378 sequences is available from the authors upon request. Evolutionary model was obtained with JModeltest v2.1.10 software (GTR+I+G) [30], chosen according the Akaike Information Criterion (AIC) and the Maximum Likelihood value (lnL), and phylogenetic trees were constructed using the Maximum likelihood method with RAxML v7.0 software. The robustness of the phylogenetic grouping was evaluated by bootstrap analysis with 1000 replicates. Phylogenetic tree was finally visualized in Figtree v1.4.3 software (available at http://tree.bio.ed.ac.uk/software/figtree/).

### Single nucleotide polymorphisms assessment

The presence of single nucleotide polymorphisms (SNP) of potential clinical and epidemiological relevance at ENV/NS1 regions was manually determined by comparing the nucleotide and amino acid changes in the generated sequences with the remaining sequences from As/Am genotype, employing as references Brazilian sequences obtained from the states of Rio de Janeiro and São Paulo, during 2007–2011 (GenBank accession numbers: JX286516-JX286521, JX286550/51, and MN589858-MN589884). The translation of the nucleotide sequences to amino acids was performed employing the BioEdit v7.2.5.0 software.

### Spatiotemporal dispersion analyses

Sequences generated within this work plus other 135 DENV-2 sequences obtained from the GenBank (S1 Table), also employed for phylogenetic analysis, were used, together with their corresponding epidemiological data, to carry on further temporal and geographical estimations of the evolutionary process. First, the presence of sufficient temporal signal in the dataset was evaluated with the TempEst v1.5.1 software [31], to proceed then with phylogenetic molecular clock analysis. The time scale of the evolutionary process was assessed employing the sequences collection dates obtained from the GenBank annotations, and a GTR+I+G4 nucleotide substitution model, a relaxed uncorrelated lognormal molecular clock model, and a Bayesian Skyline coalescent tree prior. Migration events and their most reliable migration pathways were estimated using a non-reversible discrete phylogeography model with the Bayesian stochastic search variable selection approach, and a continuous-time Markov chain (CTMC) migration rate reference prior. The analysis was implemented in BEAST v1.8.1 software package [32]. The Markov Chain Monte Carlo analysis was run for 100 million generations and convergence was assessed in Tracer v1.7 (http://tree.bio.ed.ac.uk/software/tracer): 10% of the sampling trees were discarded as burn-in and accepted effective sample sizes (ESS) values should be higher than 200. The 95% highest posterior density (HPD95%) interval was considered to estimate uncertainty for each estimated parameter. A maximum clade credibility tree (MCCT) was constructed with the TreeAnnotator v1.8.1 software (part of the BEAST package) after discarding 10% of the sampling, and visualized with the FigTree v1.4.3 program (available at http://tree.bio.ed.ac.uk/soft-ware/figtree/).

## Results and discussion

All of the ten analyzed samples tested positive for DENV-2 through the RT-PCR under Lanciotti´s protocol. In general, they all belonged to adults' patients between 23 and 61 years old, being 6 males and 4 females. None of them presented clinical signs of severity. Their respective

epidemiological characteristics can be found in S2 Table. Two out of this ten samples were selected for DENV-2 genetic sequencing based on their epidemiological characteristics: sample number 80, belonging to a 38 years-old female patient from the district of Vassouras who manifested the infection in mid-January, and sample number 230, belonging to a 37 years-old male patient from the district of Volta Redonda manifesting dengue fever in mid-March.

Phylogenetic analysis performed over the two generated sequences showed that DENV-2 strain detected in both patients sera belongs to the same lineage of As/Am genotype that caused the outbreak in 2008. However, it grouped in a totally separated cluster from sequences previously obtained from different locations of Brazil (Fig 1). The current lineage has been previously described by Mir et al. as lineage IV, which dominated the epidemics in South and Central America during the 2000s [33].

Surprisingly, this year´s sequences grouped together with sequences from Puerto Rico of 2005–2007, in a highly supported cluster (Fig 1). These findings suggest that a new viral strain may have been introduced into the state of Rio de Janeiro, which in effect, is genetically different from viral strains that kept on circulating basally in other regions of the country, at least in the viral covered region under study.

Based on phylogenetic results, we proceeded to identify the genetic positions on viral sequences involved in genetic distance between the viral isolates under study and the remaining Brazilian ones from lineage IV. Twenty-four different SNPs were detected, being 14 on the partial sequenced ENV region (genetic positions 1482–2421) and 10 on the partial NS1 (genetic positions 2422–3107) (S3 Table). Only five of them were non-synonymous, provoking an amino acid change on the viral proteins: A2023G+G2024C-S643A and C2276T-A727V on ENV; A2434G-I780V and C2878A-L928M on NS1. To notice, simultaneous nucleotide substitutions on positions 2023 and 2024 were detected exclusively in the cluster conformed by sequences generated in this work and from Puerto Rico. Furthermore, substitution C2878A-L928M was encountered only in our sequences and C2276T-A727V only in one sequence within lineage III from As/Am genotype, while A2434G-I780V was widely detected among sequences within lineage IV, and other As/Am lineages and genotypes as well. However, it was absent in the ones representing Brazilian epidemic strain from 2008. No known effect has been found in literature for any of these substitutions. On the other hand, among the 19 synonymous substitutions, three of them were unique for our sequences: G1710T, A1995G and C2307T, located within the ENV. Conversely, from the remaining 16, only half was detected in sequences from lineage IV. However, none of them among the ones from the 2008 strain. The other half was detected in sequences of other As/Am lineages and/or genotypes (S3 Table). In short, all these observations lead us to suspect that the viral strain circulating currently in the state of Rio de Janeiro may have indeed, a different origin than the one that caused the outbreak in 2008 and spread to other regions of the country, mainly through the Southeast.

Another noteworthy fact is that 2019 cases presented different clinical-epidemiological characteristics in contrast with 2008´s. During the first semester of 2008, 209,309 cases were confirmed, including 10947 hospitalizations and 230 deceases, affecting mainly the age group of children under 15 years old [34]. During the same period by 2019, 27,913 probable dengue cases were notified in the state of Rio de Janeiro, of which 59.6% were confirmed clinical and laboratory. Only 267 of them required hospitalization, and no deceases have been yet reported. Most cases occurred in young adults between 20 and 49 years old, with no clear predominance of any gender [35]. Likewise, samples analyzed in this study, corresponded to young adults' patients, both presenting a classic dengue fever (Clinical end epidemiological characteristics are available in S2 Table). It is important to mention that also during 2019, a chikungunya outbreak of great magnitude is scourging the state of Rio de Janeiro [35]. It could be likely, that vector competence by both viruses may be leading to a lower circulation of DENV-2, and thus,

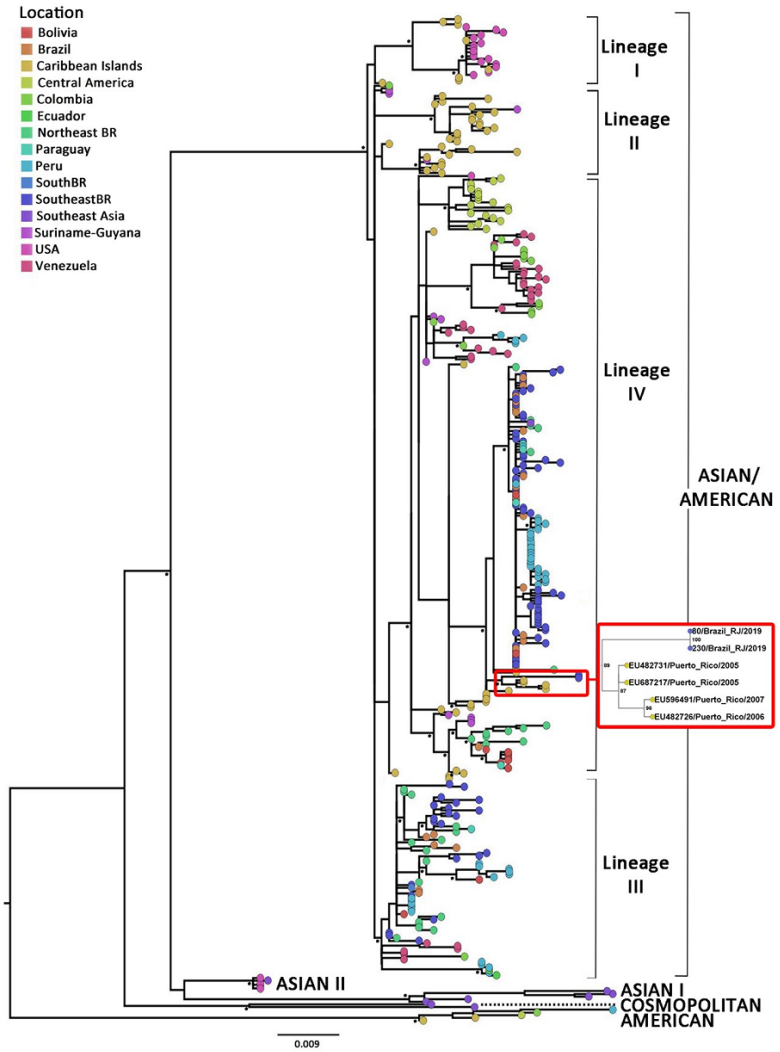


**Fig 1. Maximum likelihood phylogenetic tree of DENV-2 partial envelope and NS1 coding sequences.** DENV-2 dataset includes partial sequences (positions 1482–3107) principally from the As/Am (n = 361), American (n = 5, from Colombia, Peru, El Salvador and Caribbean Islands), Asian I (n = 5, from Southeast Asia [Thailand and Cambodia]), Asian II (n = 5, from the United States of America [USA] and Southeast Asia [Papua New Guinea]) and Cosmopolitan (n = 2, from Southeast Asia [India and Singapore]) genotypes. As/Am genotype includes sequences from USA (n = 19), Central America [Mexico (n = 5), Nicaragua (n = 13), Guatemala (n = 1), Belize (n = 1), El Salvador (n = 1)], Guyana (n = 1), Suriname (n = 11), Ecuador (n = 2), Venezuela (n = 35), Colombia (n = 15), Peru (n = 48), Bolivia (n = 12), Paraguay (n = 5), Brazil [BR] (n = 137, which includes 77 from the southeast region and 32 from the northeast) and from the Caribbean Islands which includes Jamaica (n = 1), Puerto Rico (n = 13), Cuba (n = 2), Dominican Republic (n = 4) and the Lesser Antilles islands (n = 36) conformed by Trinidad and Tobago, the Virgin Islands, Aruba, Dominica, Barbados, Grenada, Martinique, among others. Four main lineages (I to IV) are identified within As/Am genotype. Taxa are represented with circles and colored according to the geographic origin of each sequence as indicated at the legend (up left). The small cluster containing isolates from the state of Rio de Janeiro in 2019 is zoomed-in, and the two sequences generated in this study are indicated with names 80/Brazil_RJ/2019 and 230/Brazil_RJ/2019. The asterisk in the nodes corresponds to bootstrap values higher than 70, obtained with 1000 replicates. The scale bar indicates the genetic distances. The branch lengths are drawn to scale with the bar at the bottom indicating nucleotide substitutions per site.

https://doi.org/10.1371/journal.pone.0225879.g001

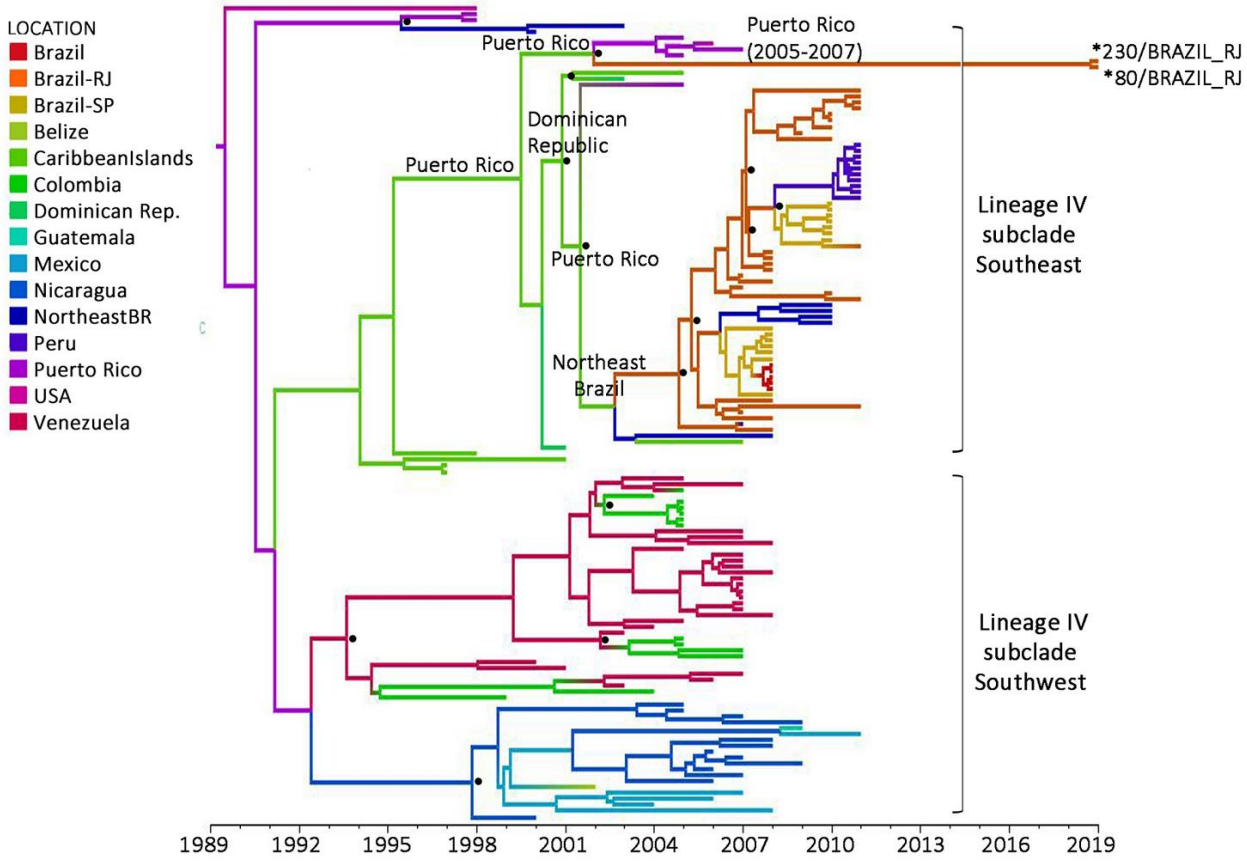


**Fig 2. Time-scaled Bayesian maximum clade credibility tree corresponding to the lineage IV of As/Am genotype.** Two major highly supported subclades are identified: southeast and southwest. Brazilian sequences are all included in the southeast subclade, and isolates under study are highlighted with an asterisk (*). Branches are colored according to the most probable location (legend shown on the left side) of their parental node inferred by discrete phylogeographical analysis. Location at key nodes involved in the introduction of subclade southeast into Brazil are highlighted. Posterior state probability values higher than 0.7 at key nodes are represented by a dot. All horizontal branch lengths are drawn to a scale of years. Time scale can be observed in the x-axis. The tree is automatically rooted under the assumption of a relaxed molecular clock. In the color-legend, Caribbean Islands includes the countries of Jamaica, Cuba, Virgin Islands, Martinique and Saint Kitts and Nevis.

https://doi.org/10.1371/journal.pone.0225879.g002

a reduced number of cases. This scenario may be influencing the marked contrast between the total number of cases.

Considering the phylogenetic results, and to have a more detailed picture of where this new DENV-2 strain comes from, we conducted temporal and geographical analysis with a reduced dataset of 137 sequences conforming lineage IV group of As/Am genotype. Sequences within this cluster are indicated in the S1 Table. They exhibited a positive correlation between genetic divergence and sampling time ($R^2 = 0.6824$) and appeared to be suitable for phylogenetic molecular clock analysis, which was after that, implemented in BEAST package [32], as described in the above section. These results suggest that the viral strain detected in our study originated in Puerto Rico (Posterior state probability [PSP] = 0.81) in 2002 (95% HPD: 2000–2004) (Fig 2), and was then introduced in our state, where it started to spread in late 2018 (95% HPD: 2018–2019). To notice, no DENV-2 case has been diagnosed during the course of 2019 in the Caribbean islands [36], which clearly suggests that Rio de Janeiro´s 2019 strain could have been circulating elsewhere, up to its arrival into the state, or could have been replicating silently for some time up to rise to a detectable level. It would be likely that actually, the

viral strain that entered into the state of Rio de Janeiro might well be introduced first into a different Brazilian state and spread then to Rio de Janeiro, based on the increasing DENV-2 reported cases of other states. As previously mentioned, since 2017, DENV-2 cases have been increasing and spreading through the central-west region, reaching the southeast region in 2018 and early 2019. The latter was responsible for 65.7% of the cases reported until March 2019 and is witnessing local epidemics in several municipalities of three of the four states that integrate it: São Paulo, Minas Gerais, and Espírito Santo; coincidentally, the three states that surround geographically the fourth integrant of the group, the state of Rio de Janeiro [26]. Nevertheless, no molecular epidemiology analyses have been carried on to study genetic viral characteristics on those cases, and to confirm this statement we will need further sequencing yet unavailable. In fact, important caveats should be taken into account for the posed hypothesis: the limited number of available sequences of Latin America covering the region under study, and the lack of Brazilian sequences from this last 5 years period. These two aspects, if different, could be responsible for dissimilar estimations. The lack of DENV-2 Brazilian sequences over recent times goes hand-in-hand with the low circulation of this serotype across the country. Nevertheless, filling this information gap would be determinant to define when this new strain has actually entered into our territory.

Even though it is not the main focus of this note, the spatial and temporal origin of lineage IV estimated in our analysis is consistent with results obtained by other groups [15, 33]. According to our calculations, this lineage probably arose in Puerto Rico in middle 1989 (95% HPD = 1985–1993) and became the dominant lineage in South and Central America from the early/middle 2000s onwards. The subclade that spread through the southeast pathway, probably arose with the introduction of a virus from Puerto Rico (PSP = 0.67) into the northeast region of Brazil around 2003 (95% HPD = 2001–2004), moving then to the state of Rio de Janeiro (PSP = 0.65) in 2005 (95% HPD = 2004–2006), and spreading thereafter through the southeast region. This northeast passage, however, presented a low probability (PSP = 0.3). Drumond and collaborators discussed in their work this possible migration pathway through the north as well. Nevertheless, their observation was statistically unsupported [15]. For Mir and collaborators, however, the circulation of this Southeast subclade, named in their work IV-SA4, started after the introduction of the virus from the Great Antilles directly into the southeast Brazilian region in 2004, moving then to the northeastern region, as well as to other countries in South America [33]. The similarities detected between our work and those already mentioned, give extra credibility to the estimates made about this new viral strain detected in the state of Rio de Janeiro.

According to our estimations, the evolutionary rate of lineage IV was $9.5x10^{-4}$ substitutions/site/year (95% HPD = $7.7x10^{-4}$–$1.2x10^{-3}$ substitutions/site/year), exactly the same reported by Mir et al [33]. To notice, the strain giving rise to the virus isolates detected this year in the state of Rio de Janeiro, presented a nucleotide substitution rate at the lower limit of the 95% HPD interval, which would be consistent with slower evolution dynamics and a delayed detection.

On the other hand, our results regarding DENV-2 population dynamic of lineage IV showed a clear drop in population size between 2005 and 2010, and remained steady until this year (Fig 3). This analysis is representing the lineage IV as a whole, including not only the Brazilian strains but the remaining from Latin America too. The dropping and posterior steady demographic behavior of lineage IV could be consistent with the fact that other DENV serotypes and arboviruses like ZIKV and CHIKV have been responsible for the main epidemics of these last years in the American continent [37–39]. Furthermore, the four DENV serotypes are circulating in the Americas, and have been circulating simultaneously in several countries [40]. Even though it may be difficult to trace the exact history of each DENV serotype in the

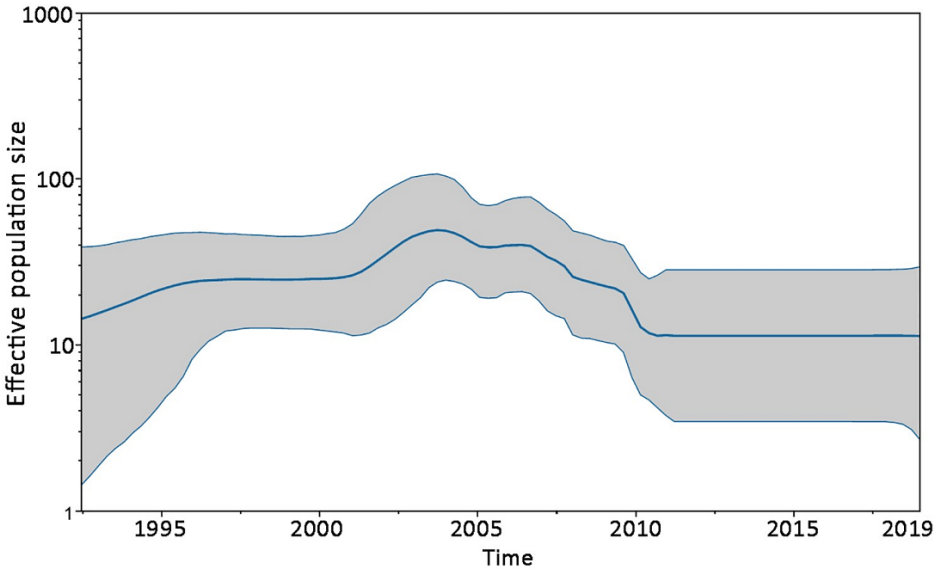


**Fig 3. Demographic reconstruction of lineage IV of As/Am genotype.** Changes in effective population size since the time of the most recent common ancestor were estimated using the uncorrelated lognormal relaxed molecular clock and the Bayesian Skyline coalescent model. Middle blue line represented the mean value for the effective sample size, while grey areas the 95% confidence interval.

https://doi.org/10.1371/journal.pone.0225879.g003

whole Latin America, it is visible that more than 55% of the total dengue cases notified by the Pan-American Health Organization (PAHO) belonged to Brazil [41]. This means that in a certain way, Latin American DENV history is capturing the Brazilian one. In this regard, and combined with epidemiological data [37, 41–42], it can be suspected that beyond particular places, DENV-2 lineage IV has not been predominant in the Americas since around 2000, which potentially reflects on its demographic reconstruction dynamics.

The phenomenon of DENV-2 lineage replacement across successive epidemic outbreaks has been well demonstrated by Mir and collaborators [33]. It should be considered that the circulation of lineage III (that dominated the Caribbean and South America in the 1990s) and IV in the state of Rio de Janeiro, was separated by an 8-year period. This might mean that, aside from the potential viral differences between both lineages itself [16], a generation of children born during this inter-lineage silent period had never be exposed to this serotype, by the time lineage IV entered the state. This fact could be somehow involved in the changes observed in 2007–2008 outbreak regarding the age-group affected.

In effect, a recent PAHO web bulletin is warning Latin American countries about a particular characteristic of the current dengue epidemic, which is threatening not only Brazil but the whole region too: DENV-2 seems to be affecting mostly children and adolescents under the age of 15. In Guatemala, they represent 52% of total cases of severe dengue, while in Honduras, they constitute 66% of all confirmed deaths. Probably, this may be due to their lack of immunity, a consequence of their shorter age and less exposure to the virus in the past [43]. This behavior has not yet been observed in Brazil. However, the current evidence and the learnings from the 2008 outbreak give clear indications that surveillance and control of the current epidemic need to be strengthened.

The facts provided by our work combined with the remaining pieces of evidence, seek to contribute with the health department and medical workers in the outbreak response, emphasizing the necessity of active disease surveillance, including laboratory diagnosis, vector

control, and healthcare professionals training for appropriate clinical diagnosis and clinical management of patients with dengue. Working together, we may then be able to ensure a response against the current DENV-2 epidemic scenario, attempting to prevent the outbreak from being of significant magnitude like the one the state of Rio de Janeiro has already experienced a decade ago.

## Supporting information

**S1 Table. GenBank accession number, country of origin, and year of isolation of sequences included in phylogenetic and temporal/geographical analysis.**
(XLSX)

**S2 Table. Clinical and epidemiological information about cases under study.**
(XLSX)

**S3 Table. Single nucleotide polymorphism (SNP) detected in both sequenced samples and its presence within other lineages or genotypes.**
(XLSX)

## Author Contributions

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**Data curation:** María Celeste Torres.

**Formal analysis:** María Celeste Torres.

**Funding acquisition:** Ana María Bispo de Filippis.

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**Visualization:** María Celeste Torres, Fernanda de Bruycker Nogueira, Ana María Bispo de Filippis.

**Writing – original draft:** María Celeste Torres, Fernanda de Bruycker Nogueira.

**Writing – review & editing:** María Celeste Torres, Ana María Bispo de Filippis.

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Supplementary Information

**S1 Table.** GenBank accession number, country of origin, and year of isolation of sequences included in phylogenetic and temporal/geographical analysis.

| Genbank sequenced employed for phylogenetic analyses | | | Lineage IV As/Am Gt- subset for temporal and geographical analysis |
|---|---|---|---|
| GenBank Accession Number | Sequence Location | Year of Collection | |
| MN589879 | Brazil_RJ | 1999 | |
| MN589880 | Brazil_RJ | 1999 | |
| MN589881 | Brazil_RJ | 1999 | |
| MN589882 | Brazil_RJ | 2000 | |
| MN589883 | Brazil_RJ | 2001 | |
| MN589884 | Brazil_RJ | 2001 | |
| MN589858 | Brazil_RJ | 2007 | Yes |
| MN589859 | Brazil_RJ | 2007 | Yes |
| MN589860 | Brazil_RJ | 2007 | Yes |
| MN589861 | Brazil_RJ | 2008 | Yes |
| MN589862 | Brazil_RJ | 2008 | Yes |
| MN589863 | Brazil_RJ | 2008 | Yes |
| MN589864 | Brazil_RJ | 2008 | Yes |
| MN589865 | Brazil_RJ | 2008 | Yes |
| MN589866 | Brazil_RJ | 2009 | Yes |
| MN589867 | Brazil_RJ | 2010 | Yes |
| MN589868 | Brazil_RJ | 2010 | Yes |
| MN589869 | Brazil_RJ | 2010 | Yes |
| MN589870 | Brazil_RJ | 2010 | Yes |
| MN589871 | Brazil_RJ | 2010 | Yes |
| MN589872 | Brazil_RJ | 2011 | Yes |
| MN589873 | Brazil_RJ | 2011 | Yes |
| MN589874 | Brazil_RJ | 2011 | Yes |
| MN589875 | Brazil_RJ | 2011 | Yes |
| MN589876 | Brazil_RJ | 2011 | Yes |
| MN589877 | Brazil_RJ | 2011 | Yes |
| MN589878 | Brazil_RJ | 2011 | Yes |
| MK972824 (Sample230) | Brazil_RJ | 2019 | Yes |
| MK972823 (Sample80) | Brazil_RJ | 2019 | Yes |
| AB122022 | Dominican_Republic | 2001 | Yes |
| AF100466 | Venezuela_Aragua | 1990 | |
| AF208496 | Martinique | 1998 | Yes |
| AF363078 | Venezuela | - | |
| AF363092 | Venezuela | - | |
| AF398106 | Venezuela | 1999 | |
| AF398108 | Venezuela | 1999 | |
| AF398113 | Venezuela | 2000 | |

| | | |
|---|---|---|
| AF489932 | Brazil | 2001 |
| AY158329 | Venezuela | 1990 |
| AY484609 | Suriname | 1993 |
| AY484610 | Suriname | 1993 |
| AY484611 | Bolivia | 1997 |
| AY484612 | Suriname | 1999 |
| AY484613 | Suriname | 1993 |
| AY484614 | Suriname | 1999 |
| AY484615 | TrinidadAndTobago | 1997 |
| AY484616 | SaintLucia | 1999 |
| AY484617 | SaintLucia | 1999 |
| AY484618 | TrinidadAndTobago | 1997 |
| AY484619 | Aruba | 1996 |
| AY484620 | Barbados | 1998 |
| AY484621 | SaintLucia | 1999 |
| AY484622 | Curacao | 1996 |
| AY484623 | SaintVincentandGrenadines | 1998 |
| AY484624 | Dominica | 1995 |
| AY484625 | Dominican_Republic | 1984 |
| AY484626 | Dominican_Republic | 1990 |
| AY484627 | Dominica | 1995 |
| AY484628 | SaintVincentandGrenadines | 1998 |
| AY484629 | Grenada | 1995 |
| AY484630 | SaintVincentandGrenadines | 1998 |
| AY484631 | SaintLucia | 1999 |
| AY484632 | TrinidadAndTobago | 2000 |
| AY484633 | TrinidadAndTobago | 1997 |
| AY484634 | Suriname | 1986 |
| AY484635 | Suriname | 1986 |
| AY484636 | SaintVincentandGrenadines | 1998 |
| AY484637 | Suriname | 1986 |
| AY484638 | Bahamas | 1989 |
| AY484639 | TrinidadAndTobago | 1986 |
| AY484640 | TrinidadAndTobago | 1987 |
| AY484641 | TrinidadAndTobago | 1988 |
| AY484642 | Guyana | 2000 |
| AY484643 | TrinidadAndTobago | 1986 |
| AY484644 | Colombia | 1993 |
| AY484645 | TrinidadAndTobago | 1989 |
| AY484646 | TrinidadAndTobago | 1996 |
| AY484647 | TrinidadAndTobago | 1986 |
| AY484648 | Barbados | 1987 |
| AY484649 | Ecuador | 2000 |
| AY484650 | Barbados | 1988 |
| AY484651 | Ecuador | 2000 |
| AY484652 | Suriname | 1986 |
| AY484653 | TrinidadAndTobago | 2000 |

| | | | |
|---|---|---|---|
| AY484654 | Suriname | 1993 | |
| AY484655 | Venezuela | 1990 | |
| AY484656 | Dominica | 1995 | |
| AY484657 | TrinidadAndTobago | 1996 | |
| AY484658 | Curacao | 1993 | |
| AY484659 | TrinidadAndTobago | 1986 | |
| AY484660 | Suriname | 1999 | |
| AY484666 | El_Salvador | 1987 | |
| AY484667 | TrinidadAndTobago | 1981 | |
| AY702036 | Cuba | 1997 | Yes |
| AY702038 | Cuba | 1997 | Yes |
| DQ181804 | Thailand | 1984 | |
| DQ181805 | Thailand | 1979 | |
| EU045312 | Paraguay | 2005 | |
| EU045313 | Paraguay | 2005 | |
| EU056811 | Peru_Iquitos | 1995 | |
| EU482544 | USA | 2006 | |
| EU482545 | Puerto_Rico | 1998 | Yes |
| EU482548 | USA | 2006 | |
| EU482550 | Puerto_Rico | 1998 | |
| EU482551 | USA | 2006 | |
| EU482553 | USA | 2006 | |
| EU482560 | USA | 1998 | Yes |
| EU482604 | Venezuela | 2007 | Yes |
| EU482605 | Venezuela | 2007 | Yes |
| EU482606 | Venezuela | 2007 | Yes |
| EU482607 | Venezuela | 2007 | Yes |
| EU482608 | Venezuela | 2007 | Yes |
| EU482723 | USA | 2002 | |
| EU482724 | Puerto_Rico | 2005 | |
| EU482725 | USA | 2006 | |
| EU482726 | Puerto_Rico | 2006 | Yes |
| EU482731 | Puerto_Rico | 2005 | Yes |
| EU482735 | Puerto_Rico | 1998 | Yes |
| EU596488 | USA | 2007 | |
| EU596489 | USA | 2007 | |
| EU596490 | Puerto_Rico | 2007 | |
| EU596491 | Puerto_Rico | 2007 | Yes |
| EU677141 | Puerto_Rico | 1996 | |
| EU677144 | Puerto_Rico | 1999 | |
| EU677146 | USA | 2004 | |
| EU687214 | USA | 2004 | |
| EU687216 | Puerto_Rico | 2005 | Yes |
| EU687217 | Puerto_Rico | 2005 | Yes |
| EU687220 | Venezuela_Aragua | 1995 | |
| EU687224 | Puerto_Rico | 2000 | |
| EU687230 | USA | 2001 | |

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| EU687232 | Puerto_Rico | 2001 | |
| EU687235 | USA | 2003 | |
| EU687236 | USA | 2003 | |
| EU687238 | USA | 2003 | |
| EU687241 | USA | 2003 | |
| EU687245 | USA | 2005 | |
| EU781135 | USA | 2005 | |
| EU854294 | Colombia | 2005 | Yes |
| FJ024473 | Colombia | 2005 | Yes |
| FJ024474 | Colombia | 2005 | Yes |
| FJ024475 | Colombia | 2005 | Yes |
| FJ024477 | Colombia_Antioquia | 2004 | Yes |
| FJ182012 | Colombia | 2005 | Yes |
| FJ639705 | Cambodia | 2003 | |
| FJ639732 | Venezuela | 2005 | Yes |
| FJ639733 | Venezuela | 2005 | Yes |
| FJ639734 | Venezuela | 2003 | Yes |
| FJ639783 | Venezuela_Aragua | 2003 | Yes |
| FJ639822 | Venezuela_Aragua | 2006 | Yes |
| FJ850072 | NorthernBR | 2000 | |
| FJ850074 | NorthernBR | 2001 | |
| FJ850076 | NorthernBR | 2002 | |
| FJ850078 | NorthernBR | 2003 | |
| FJ850082 | NorthernBR | 2004 | |
| FJ850085 | NorthernBR | 2005 | |
| FJ850088 | NorthernBR | 2006 | |
| FJ850091 | NorthernBR | 2007 | Yes |
| FJ850105 | Venezuela | 2007 | Yes |
| FJ850106 | Venezuela | 2008 | Yes |
| FJ850107 | Venezuela | 2008 | Yes |
| FJ850108 | Venezuela | 2008 | Yes |
| FJ850112 | Venezuela_Caracas | 2004 | Yes |
| FJ898451 | Dominican_Republic | 2003 | Yes |
| FJ898452 | Thailand | 2003 | |
| FJ898453 | Virgin_Islands | 2005 | Yes |
| FJ898460 | SaintKitts_NevisSaintKitts | 2001 | Yes |
| FJ898461 | Belize | 2002 | Yes |
| FJ898465 | Venezuela_Aragua | 1998 | |
| FJ898466 | Venezuela_Aragua | 2000 | Yes |
| FJ898467 | Venezuela_Aragua | 2005 | Yes |
| FJ906959 | PapuaNewGuinea | 2008 | |
| GQ199890 | NorthernBR | 2008 | Yes |
| GQ199892 | Jamaica | 2007 | Yes |
| GQ330472 | Brazil_RP_RMB | 2009 | |
| GQ368158 | Brazil | 1998 | |
| GQ368159 | Brazil | 1998 | |
| GQ368160 | Brazil | 1998 | |

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|---|---|---|---|
| GQ368161 | Brazil | 2007 | |
| GQ368162 | Brazil | 2007 | |
| GQ368163 | Brazil | 2007 | |
| GQ368164 | Brazil | 2007 | |
| GQ368165 | Brazil | 2008 | |
| GQ368166 | Brazil | 2008 | |
| GQ368167 | Brazil | 2008 | |
| GQ368168 | Brazil | 2008 | |
| GQ368169 | Brazil | 2008 | |
| GQ368170 | Brazil | 2008 | |
| GQ368171 | Brazil | 2008 | |
| GQ368172 | Brazil | 2008 | |
| GQ368173 | Brazil | 1998 | |
| GQ368174 | Brazil | 1998 | |
| GQ368175 | Brazil | 2008 | |
| GQ368176 | Brazil | 2008 | |
| GQ868516 | Mexico_Yucatan | 2007 | Yes |
| GQ868549 | Brazil_SP | 2008 | Yes |
| GQ868550 | Brazil | 2008 | Yes |
| GQ868551 | Brazil_SP | 2008 | Yes |
| GQ868552 | Colombia_Santander | 1998 | |
| GQ868553 | Colombia_Santander | 1999 | Yes |
| GQ868554 | Colombia_Santander | 2004 | Yes |
| GQ868555 | Colombia | 2005 | Yes |
| GQ868556 | Colombia | 2005 | Yes |
| GQ868557 | Colombia | 2005 | Yes |
| GQ868558 | Colombia | 2007 | Yes |
| GQ868592 | Colombia | 1986 | |
| GQ868640 | NorthernBR | 2003 | Yes |
| GQ868641 | Venezuela | 2007 | Yes |
| GU131864 | Brazil_SP | 2008 | Yes |
| GU131879 | Brazil | 2008 | Yes |
| GU131880 | Brazil | 2008 | Yes |
| GU131881 | Brazil_SP | 2008 | Yes |
| GU131882 | Brazil_SP | 2008 | Yes |
| GU131883 | Brazil | 2008 | Yes |
| GU131884 | Brazil_SP | 2008 | Yes |
| GU131885 | Brazil | 2008 | Yes |
| GU131902 | Cambodia | 2008 | |
| GU131947 | Colombia | 2007 | Yes |
| GU131955 | Mexico_QuintanaRoo | 2004 | Yes |
| GU131959 | Mexico_Yucatan | 2006 | Yes |
| HM181971 | Brazil_SP | 2008 | Yes |
| HM582117 | TrinidadAndTobago | 1974 | |
| HQ012508 | Brazil_BA | 1991 | |
| HQ012509 | Brazil_CE | 1994 | |
| HQ012510 | Brazil_RJ | 1995 | |

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|---|---|---|---|
| HQ012511 | Brazil_RJ | 1995 | |
| HQ012512 | Brazil_RS | 1996 | |
| HQ012513 | Brazil_BA | 1996 | |
| HQ012514 | Brazil_RN | 1997 | |
| HQ012515 | Brazil_RN | 1997 | |
| HQ012516 | Brazil_RJ | 1998 | |
| HQ012517 | Brazil_RJ | 1999 | |
| HQ012518 | Brazil_RJ | 2000 | |
| HQ012519 | Brazil_RJ | 2000 | |
| HQ012520 | Brazil_RJ | 2001 | |
| HQ012521 | Brazil_RJ | 2001 | |
| HQ012522 | Brazil_RJ | 2002 | |
| HQ012523 | Brazil_ES | 2002 | |
| HQ012524 | Brazil_ES | 2003 | |
| HQ012525 | Brazil_RJ | 2007 | |
| HQ012526 | Brazil_RJ | 2007 | |
| HQ012527 | Brazil_RJ | 2008 | |
| HQ012528 | Brazil_RJ | 2008 | |
| HQ012529 | Brazil_BA | 2009 | |
| HQ012530 | Brazil_ES | 2009 | |
| HQ012531 | Brazil_RJ | 2010 | |
| HQ012532 | Brazil_RJ | 2010 | |
| HQ012533 | Brazil_RJ | 1990 | |
| HQ012534 | Brazil_RJ | 1991 | |
| HQ012535 | Brazil_CE | 1994 | |
| HQ012536 | Brazil_RJ | 1998 | |
| HQ012537 | Brazil_RJ | 1999 | |
| HQ012538 | Brazil_RJ | 1990 | |
| HQ026763 | Brazil_RJ | 2008 | Yes |
| HQ332185 | Venezuela | 2007 | Yes |
| HQ332186 | Venezuela | 2007 | Yes |
| HQ332187 | Venezuela | 2007 | Yes |
| HQ332189 | Venezuela | 2007 | Yes |
| HQ332190 | Venezuela | 2007 | Yes |
| HQ541786 | Nicaragua_Managua | 2006 | Yes |
| HQ541787 | Nicaragua_Managua | 2007 | Yes |
| HQ541788 | Nicaragua_Managua | 2007 | Yes |
| HQ541792 | Nicaragua_Managua | 2008 | Yes |
| HQ541793 | Nicaragua_Managua | 2005 | Yes |
| HQ541794 | Nicaragua_Managua | 2005 | Yes |
| HQ541798 | USA_California | 2009 | |
| HQ541799 | USA_California | 2010 | |
| HQ705624 | Nicaragua_Managua | 2009 | Yes |
| HQ733861 | Nicaragua_Managua | 2006 | Yes |
| HQ999999 | Guatemala | 2009 | Yes |
| JF327392 | Singapore | 2009 | |
| JF357906 | Nicaragua_Managua | 2008 | Yes |

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|---|---|---|---|
| JF730051 | Nicaragua_Managua | 2009 | Yes |
| JF730053 | USA_California | 2006 | |
| JF730054 | USA_California | 2009 | |
| JN819407 | Venezuela_merida | 2007 | Yes |
| JN819408 | Venezuela_aragua | 2001 | Yes |
| JN819416 | Nicaragua_Managua | 2000 | Yes |
| JN819419 | NorthernBR | 2000 | Yes |
| JN819421 | Nicaragua_Managua | 2007 | Yes |
| JN819422 | Mexico_Tapachula | 2008 | Yes |
| JN819424 | Nicaragua_Carazo | 2006 | Yes |
| JQ710657 | Brazil_RJ | 2011 | |
| JQ710658 | Brazil_RJ | 2010 | |
| JX051767 | Peru | 2000 | |
| JX051768 | Peru | 2000 | |
| JX051769 | Peru | 2001 | |
| JX051770 | Peru | 2001 | |
| JX051771 | Peru | 2002 | |
| JX051772 | Peru | 2002 | |
| JX051773 | Peru | 2002 | |
| JX051774 | Peru | 2002 | |
| JX051775 | Peru | 2002 | |
| JX051776 | Peru | 2002 | |
| JX051777 | Peru | 2007 | |
| JX051778 | Peru | 2007 | |
| JX051779 | Peru | 2007 | |
| JX051780 | Peru | 2008 | |
| JX051781 | Peru | 2009 | |
| JX051782 | Peru | 2009 | |
| JX051783 | Peru | 2009 | |
| JX051784 | Peru | 2009 | |
| JX051785 | Peru | 2010 | |
| JX051786 | Peru | 2010 | |
| JX051787 | Peru | 2010 | |
| JX051788 | Peru | 2010 | |
| JX051789 | Peru | 2010 | |
| JX051790 | Peru | 2010 | |
| JX051791 | Peru | 2010 | |
| JX051792 | Peru | 2010 | |
| JX051793 | Peru | 2011 | |
| JX051794 | Peru | 2011 | |
| JX051795 | Peru | 2011 | |
| JX051796 | Peru | 2011 | |
| JX051797 | Peru | 2011 | |
| JX051798 | Peru | 2011 | |
| JX051801 | Bolivia | 2003 | |
| JX051802 | Bolivia | 2007 | |
| JX051803 | Bolivia | 2007 | |

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|---|---|---|---|
| JX051804 | Bolivia | 2007 | |
| JX051805 | Bolivia | 2007 | |
| JX051806 | Bolivia | 2007 | |
| JX051807 | Bolivia | 2010 | |
| JX051808 | Bolivia | 2006 | |
| JX051809 | Bolivia | 2007 | |
| JX051810 | Bolivia | 2010 | |
| JX051811 | Bolivia | 2010 | |
| JX051812 | Paraguay | 2010 | |
| JX051813 | Paraguay | 2010 | |
| JX051814 | Paraguay | 2010 | |
| JX073928 | Brazil | 2001 | |
| JX286516 | Brazil_SP | 2010 | Yes |
| JX286517 | Brazil_SP | 2010 | Yes |
| JX286518 | Brazil_SP | 2010 | Yes |
| JX286519 | Brazil_SP | 2010 | Yes |
| JX286520 | Brazil_SP | 2010 | Yes |
| JX286521 | Brazil_SP | 2010 | Yes |
| JX286526 | Brazil_SP | 2010 | Yes |
| JX567950 | Brazil_RJ | 2008 | Yes |
| JX567951 | Brazil_RJ | 2008 | Yes |
| JX669476 | Brazil_PE | 2010 | Yes |
| JX669477 | Brazil_PE | 2010 | Yes |
| JX669478 | Brazil_PE | 2010 | Yes |
| JX669479 | Brazil_PE | 2010 | Yes |
| JX669480 | Brazil_PE | 1995 | |
| JX669481 | Brazil_PE | 1995 | |
| JX669482 | Brazil_PE | 1995 | |
| JX669483 | Brazil_PE | 1997 | |
| JX669484 | Brazil_PE | 1998 | |
| JX669485 | Brazil_PE | 1998 | |
| JX669486 | Brazil_PE | 1999 | |
| JX669487 | Brazil_PE | 2000 | |
| JX669488 | Brazil_PE | 2002 | |
| KC294204 | Peru_Iquitos | 2011 | Yes |
| KC294205 | Peru_Iquitos | 2011 | Yes |
| KC294206 | Peru_Iquitos | 2011 | Yes |
| KC294207 | Peru_Iquitos | 2011 | Yes |
| KC294209 | Peru_Iquitos | 2011 | Yes |
| KC294210 | Peru_Iquitos | 2011 | Yes |
| KC294211 | Peru_Iquitos | 2011 | Yes |
| KC294212 | Peru_Iquitos | 2011 | Yes |
| KC294214 | Peru_Iquitos | 2011 | Yes |
| KC294218 | Peru_Iquitos | 2011 | Yes |
| KC847991 | Peru | 2011 | |
| KC847992 | Peru | 2012 | |
| KC847993 | Peru | 2012 | |

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|---|---|---|---|
| KC847994 | Peru | 2012 | |
| KC847995 | Peru | 2012 | |
| KC847996 | Peru | 2012 | |
| KJ189370 | Mexico | 2011 | Yes |
| KP188555 | Brazil_SJRP | 2013 | |
| KP188569 | Brazil_SJRP | 2014 | |
| KT438610 | Brazil_SJRP | 2013 | |
| KT438611 | Brazil_SJRP | 2013 | |
| KT438612 | Brazil_SJRP | 2013 | |
| KT438613 | Brazil_SJRP | 2013 | |
| MH822951 | India | 2013 | |
| NC_001474 | Thailand | 1964 | |

Notes: RJ: State of Rio de Janeiro; SP: State of São Paulo; SJRP: City of São Jose do Rio Preto; PE: State of Pernambuco; CE: State of Ceará; BA: State of Bahia; ES: State of Espírito Santo; RN: State of Rio Grande do Norte; RS: State of Rio Grande do Sul; RP: City of Ribeirão Preto. As/Am Gt: Asian/American genotype

**S2 Table** - Clinical and epidemiological information about cases under study. Highlighted in red, the sequenced cases

| Patient | Age (years) | Gender | District | Days of symptoms | Clinical signs and symptoms | Observations |
|---|---|---|---|---|---|---|
| **80** | 38 | Female | Vassouras | 3 | Fever, myalgia, vomiting, back pain | Without Pre-Existing Clinical Conditions |
| **230** | 37 | Male | Volta Redonda | 1 | Myalgia, sickness, headache, intense arthralgia | Without Pre-Existing Clinical Conditions |
| 31 | 60 | Male | Vassouras | 3 | Fever, myalgia, headache, sickness and vomiting, back pain | HBP |
| 72 | 42 | Male | Vassouras | 4 | Not detailed, but reported as classic dengue fever | ND |
| 3 | 61 | Female | Vassouras | 3 | Not detailed, but reported as classic dengue fever | ND |
| 65 | 26 | Female | Vassouras | 3 | Fever, myalgia, exanthema, headache, intense arthralgia, retro-orbital pain | Without Pre-Existing Conditions |
| 651 | 59 | Male | Volta Redonda | 3 | Not detailed, but reported as classic dengue fever | ND |
| 502 | 23 | Female | Volta Redonda | 1 | Fever, sickness, headache, intense arthralgia | Chronic kidney disease |
| 56 | 58 | Male | Mangaratiba | 0 | Not detailed, but reported as classic dengue fever | ND |
| 22 | 26 | Male | Nova Iguaçu | 0 | Fever, myalgia, intense arthralgia | Without Pre-Existing Conditions |

Notes: HBP= High blood pressure, ND= Not described.

**S3 Table** - Single nucleotide polymorphism (SNP) detected in both sequenced samples and its presence within other lineages or genotypes

| Genomic region (ORF position) | Nt sustitution | Aa sustitution | Brazilian seq from lineage IV- As/Am Gt | Non-brazilian seq within lineage IV - As/Am Gt | Other lineages or Genotypes |
|---|---|---|---|---|---|
| ENV (nt 937-2421) | G1641A | | - | - | Asian I Gt, As/Am Gt lineages I & II (Caribbean Islands, before 2000) |
| | G1710T | | - | - | - |
| | G1863A | | - | - | Cosmopolitan Gt (MH822951 India 2013) |
| | A1995G | | - | - | - |
| | A2023G | S643A | - | Puerto Rico (EU482726, EU482731, EU687217, EU596491) | - |
| | G2024C | | - | Puerto Rico (EU482726, EU482731, EU687217, EU596491) | - |
| | C2049T | | NorthernBR (GQ868640, JN819419, GQ199890) | Non-brazilian seq of lineage IV from Central America, Venezuela, Puerto Rico (EU482726, EU482731, EU687217, EU596491), etc. | Gts Asian I, Asian II, American, Cosmopolitan, and lineages I, II and III of As/Am Gt |
| | C2055T | | - | Nicaragua (JN819416) & Venezuela (HQ332189) | Cosmopolitan Gt (MH822951 India 2013), Asian I Gt (DQ181804 Thailand 1984), As/Am Gt lineages I & II (Caribbean Islands, before 2000) |
| | C2067T | | - | - | Asian I Gt (DQ181804 Thailand 1984), As/Am Gt lineages I & II (Caribbean Islands, before 2000) |
| | C2073T | | - | Non-brazilian seq of lineage IV from Central America, Venezuela, Colombia, etc. | Cosmopolitan Gt (MH822951 India 2013) |
| | T2247C | | NorthernBR (GQ868640, JN819419) | Non-brazilian seq of lineage IV from Central America, Cuba, Venezuela, Puerto Rico (EU482726, | Gts Asian I, Asian II, American, Cosmopolitan, and lineages I, II and III of As/Am Gt |

| | | | | | |
|---|---|---|---|---|---|
| | | | | EU482731, EU687217, EU596491), etc. | |
| | C2276T | A727V | - | - | As/Am Gt lineage III (AF489932 Brazil 2001) |
| | C2289T | | - | - | Gts Asian I, Asian II, Cosmopolitan (Southeast Asia) , As/Am Gt lineage III (GQ368160, GQ368173 Brazil 1998) |
| | C2307T | | - | - | - |
| NS1 (nt 2422-3477) | A2434G | I780V | NorthernBR (GQ868640, JN819419) | Non-brazilian seq of lineage IV from Central America, Cuba, Venezuela, Puerto Rico (EU482726, EU482731, EU687217, EU596491), etc. | Gts Asian I, Asian II, American, Cosmopolitan, and lineages I, II and III of As/Am Gt |
| | T2451C | | NorthernBR (GQ199890) | Mexico (JN819422) & Nicaragua (HQ705624) | Gts Asian I, Asian II, American, Cosmopolitan, and Colombian (GQ868552), Venezuelan (EU687220) and Brazilian sequences of lineage III - As/Am Gt |
| | G2511A | | NorthernBR (JX669497, JN819419) | Non-brazilian seq of lineage IV from Central America, Caribbean Islands, Venezuela, Puerto Rico (EU482726, EU482731, EU687217, EU596491), etc. | Gts Asian I, Asian II, American, Cosmopolitan, and lineage III of As/Am Gt |
| | A2589G | | - | - | Asian I Gt |
| | G2781A | | - | - | Gts Asian I, Asian II, American, Cosmopolitan |
| | C2878A | L928M | - | - | - |
| | T2898C | | - | Venezuela (FJ850107) | Gts Asian I, Asian II, American, Cosmopolitan |
| | C2961T | | NorthernBR (FJ850082) | - | - |
| | A2976G | | - | - | Cosmopolitan Gt (MH822951 India 2013), Asian I Gt (FJ898452 Thailand 2003) |
| | T3075C | | - | - | Cosmopolitan Gt (MH822951 India 2013), Asian II Gt |

Notes: ORF= open reading frame; ENV= envelope gene; NS1= non-structural gene 1; nt= nucleotide; Aa= amino acid; As/Am Gt= Asian/American genotype; Gt= genotype.

## 4.2 Definição da amostragem e análises de diversidade

Na base dos resultados obtidos anteriormente, foi definido que embora a nova cepa chamada de BR4 possa ser uma nova linhagem se espalhando pelo Brasil, suas sequências representativas ainda se agrupavam no clado IV do genótipo III e não eram tão geneticamente diversas da BR3. Além disso, ao contrário do que foi descrito para BR1 e BR3 (Nunes et al., 2016), não foram observadas diferenças no padrão clínico-epidemiológico entre BR3 e BR4. Assim, decidiu-se que os isolados pertencentes à linhagem BR4 fossem incluídos neste estudo.

Desta maneira, foram coletadas para o desenvolvimento deste estudo amostras de soro pertencentes aos bancos de amostras do Laboratório de Flavivírus, Centro de Referência Regional para Dengue e Febre Amarela IOC/FIOCRUZ, e de diferentes Centros de Saúde do país, coletadas de pacientes com infecção confirmada (por RT-PCR e/ou isolamento viral) de DENV-2, durante o período de 2007-2019 (Tabela 4.1). As mesmas provieram da demanda espontânea dos laboratórios, sem nenhum procedimento adicional para os pacientes, e foram mantidas armazenadas a -70°C até o seu processamento.

**Tabela 4.1** Amostras coletadas para o estudo de diversidade intra-hospedeiro.

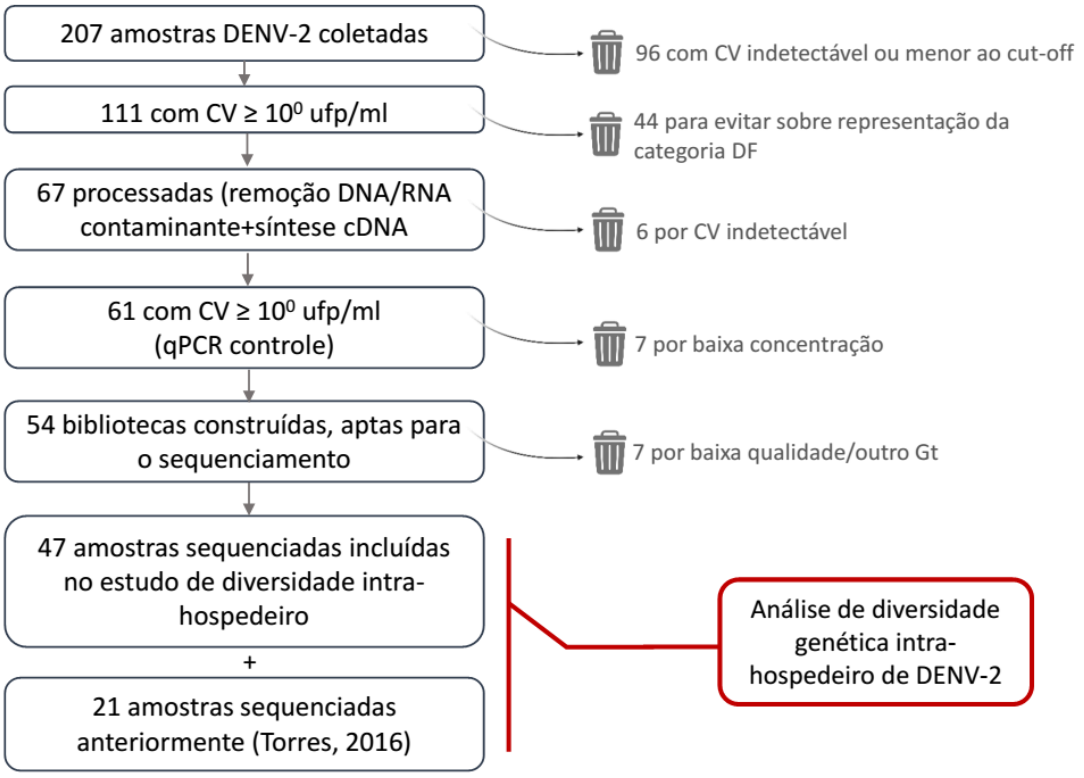
| Estado | Período (anos) | Amostras (n) | Classificação clínica da dengue (n) | Gênero (n) | Idade (faixa) | Dias de sintomas (faixa) |
|---|---|---|---|---|---|---|
| RJ (Fiocruz, LACEN) | 2008-2011 | 76 | DF: 27<br>DWS: 10<br>SD: 39 | M:41, F:35 | 7m-74 anos | 0-24 |
| | 2018-2019 | 26 | DF: 26<br>DWS: 0<br>SD: 0 | M:13, F:13 | 22-64 anos | 0-4 |
| SP (IMT) | 2010 | 45 | DF: 10<br>DWS: 18<br>SD: 17 | M:21, F:23, AI:1 | 2m-87 anos | 2-12 |
| MG (FUNED) | 2019 | 60 | DF: 58<br>DWS: 2<br>SD: 0 | M:17, F:43 | 8-71 anos | 0-4 |

RJ: estado do Rio de Janeiro, SP: estado de São Paulo, MG: estado de Minas Gerais, M: gênero masculino, F: feminino, m: meses, ND: não determinado, DF/DWS/SD: categorias da classificação clínica (WHO, 2009) que incluem os casos clássicos de febre da dengue, os casos de dengue com sinais de alarme, e os casos de dengue grave; do inglês “dengue fever/dengue with warning

signs/severe dengue" (se mantêm a mesma nomenclatura do artigo para maior claridade). As amostras procedentes do RJ provêm do Laboratório Central Noel Nutels (LACEN/RJ) e dos Laboratório de Flavivírus e de Imunologia Viral do Instituto Oswaldo Cruz, enquanto as de SP provêm do Instituto de Medicina Tropical (IMT), e as de MG da Fundação Ezequiel Dias (FUNED). Todas elas são amostras coletadas para fins diagnóstico e armazenadas em bancos de amostras próprios das instituições em questão.

Estas 207 amostras foram processadas conforme as técnicas descritas no artigo em seguida, sendo que apenas 47 conseguiram ser empregadas na análise de diversidade intra-hospedeiro, conjuntamente com as 21 amostras previamente sequenciadas (Torres, 2016). As perdas concomitantes ao próprio processamento se esquematizaram na figura 4.1, enquanto as informações especificas para cada amostra podem se achar no Anexo II.

**Figura 4.1** Seleção de amostras para a análise de diversidade intra-hospedeiro




Nota: CV: carga viral; qPCR: PCR quantitativa; DF: categoria clínica que inclui os casos clássicos de febre da dengue, do inglês "dengue fever"; Gt: genótipo.

De maneira geral, os seguintes critérios foram tidos em conta para a seleção final das amostras que compuseram o grupo de estudo especifico para a análise de diversidade intra-hospedeiro:

***Critérios de inclusão:** amostras de casos confirmados de dengue (por RT-PCR e/ou isolamento viral) pelo sorotipo viral 2 ocorridos a partir no ano 2007, cuja ficha epidemiológica informe os sinais e sintomas descritivos do quadro clínico, ou a classificação clinica já descrita segundo a guia da Organização Mundial da Saúde do ano 2009, e as datas de coleta da amostra e início dos sintomas.

***Critérios de não inclusão**: amostras de casos suspeitos de dengue não confirmados laboratorialmente para DENV-2, assim como os casos confirmados pelos sorotipos virais 1, 3, ou 4. Foram excluídas aquelas amostras de pacientes sem a ficha epidemiológica ou não preenchida corretamente, as amostras correspondentes a casos importados ao Brasil, ou pertencentes a genótipos diferentes ao III (Asiático/Americano) ou à linhagem BRI deste genótipo, assim como também aquelas cuja carga viral resultou inferior à ordem de $10^0$ ufp/ml (unidade formadora de placa por mililitro). Estima-se que por cada ufp existam entre 100 e 1000 copias do genoma viral (Richardson et al., 2006). Finalmente, foram excluídas também aquelas amostras que após o sequenciamento profundo apresentaram uma baixa qualidade ou atingissem uma profundidade menor à esperada de acordo com a suas cargas virais iniciais.

**4.2 Artigo 2** - Dengue Virus Serotype 2 Intrahost Diversity in Patients with Different Clinical Outcomes

**Relação do manuscrito com os objetivos:** Os resultados apresentados neste manuscrito são referentes aos seguintes objetivos específicos:

1) Determinar o genótipo e linhagem de DENV-2 envolvido em cada amostra, para realizar uma correta seleção das amostras que formarão parte do estudo;

2) Analisar e comparar a diversidade dos espectros de mutantes virais identificados nas amostras de DENV-2 selecionadas;

3) Descrever a relação entre o tipo de infecção (primária/secundária), quadro clínico (dengue, dengue com sinais de alarme e dengue grave) e a diversidade genética achada nas amostras de DENV-2 selecionadas;

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# Dengue Virus Serotype 2 Intrahost Diversity in Patients with Different Clinical Outcomes

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**Abstract:** Intrahost genetic diversity is thought to facilitate arbovirus adaptation to changing environments and hosts, and it might also be linked to viral pathogenesis. Dengue virus serotype 2 (DENV-2) has circulated in Brazil since 1990 and is associated with severe disease and explosive outbreaks. Intending to shed light on the viral determinants for severe dengue pathogenesis, we sought to analyze the DENV-2 intrahost genetic diversity in 68 patient cases clinically classified as dengue fever ($n$ = 31), dengue with warning signs ($n$ = 19), and severe dengue ($n$ = 18). Unlike previous DENV intrahost diversity studies whose approaches employed PCR, here we performed viral whole-genome deep sequencing from clinical samples with an amplicon-free approach, representing the real intrahost diversity scenario. Striking differences were detected in the viral population structure between the three clinical categories, which appear to be driven mainly by different infection times and selection pressures, rather than being linked with the clinical outcome itself. Diversity in the NS2B gene, however, showed to be constrained, irrespective of clinical outcome and infection time. Finally, 385 non-synonymous intrahost single-nucleotide variants located along the viral polyprotein, plus variants located in the untranslated regions, were consistently identified among the samples. Of them, 124 were exclusively or highly detected among cases with warning signs and among severe cases. However, there was no variant that by itself appeared to characterize the cases of greater severity, either due to its low intrahost frequency or the conservative effect on amino acid substitution. Although further studies are necessary to determine their real effect on viral proteins, this heightens the possibility of epistatic interactions. The present analysis represents an initial effort to correlate

DENV-2 genetic diversity to its pathogenic potential and thus contribute to understanding the virus's dynamics within its human host.

**Keywords:** dengue virus; serotype 2; intrahost diversity; severe disease

## 1. Introduction

Dengue fever (DF) is the arboviral disease with the strongest impact in terms of morbidity and mortality worldwide. Latest estimations reported that annually 390 million people around the world get infected by dengue virus (DENV), of which 96 million ultimately manifest the disease [1]. DENV infection can range from asymptomatic infection to a debilitating and potentially life-threatening acute disease in human hosts [2]. Antibody-dependent enhancement (ADE) phenomenon explains why certain cases progress to severity [3]. However, as many other hemorrhagic dengue cases occur during primary DENV infections, ADE might not be a necessary condition for the development of disease severity, which ultimately raises the question about the role of viral factors in the infection process [4]. DENVs are positive-sense, single-stranded RNA viruses that belong to the *Flaviviridae* family, genus *Flavivirus*. The DENV genome size is approximately 10.7 kb and contains a region coding for a single polyprotein flanked by a short 5′ untranslated region (UTR) and a longer 3′ UTR, both highly structured and carrying elements essential to the virus replication. The polyprotein is post-translationally cleaved into three structural proteins (capsid (C), pre-membrane/membrane (preM/M), and envelope (E)) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [5]. NS5 encodes for the replicative RNA-dependent RNA polymerase, which is a low-fidelity enzyme and thus prone to introduce genetic variability into the viral population during each cycle of RNA replication. Consequently, new viral variants get continuously generated within a single host, shaping what is defined as 'intrahost diversity' [6]. Considering that it was demonstrated for RNA viruses that just one or a few amino acid replacements within a single protein are enough to modify a particular biological feature of the virus [7,8], the intrahost diversity takes a place of high relevance on the study of the evolution of DENV populations during the course of human infection, and its relation to disease severity. Intrahost genetic diversity is thought to be advantageous for RNA viruses by facilitating their adaptation to changing environments and hosts, and as was demonstrated for many other viruses [9–12], might also influence their pathogenicity.

DENV-2 genotype III (previously named as the Asian/American genotype [13]) is circulating in Brazil since 1990 [14]. In 2008, a DENV-2 outbreak was associated with increased disease severity and a high mortality rate [15,16]. In this context, and to better understand the association of viral features with severe dengue pathogenesis, we explored DENV-2 intrahost genetic diversity in Brazilian patients with different clinical outcomes during the calendar period 2008–2019.

Several investigations attempted to determine the correlation between DENV intrahost diversity and disease severity, employing different experimental designs—from PCR+cloning+Sanger sequencing of the E gene [17,18] to PCR+Next-generation sequencing of the complete genome [19,20]. However, all of these studies employed PCR as a molecular tool, potentially generating mutational bias through amplification and primer mismatching. Our study's experimental approach consisted in an amplicon-free deep-ranging coverage of the viral genome, aiming to most reliably reflect the viral genetic variability at each stage of patient infection, to finally assess a correlation with disease severity.

## 2. Methodology

### *2.1. Ethical Statement*

This study was approved by the Oswaldo Cruz Institute Ethical Committee in Research (CAAE 90249219.6.1001.5248 number 2.998.362). All methods were performed in accordance with the World Medical Association Declaration of Helsinki.

### *2.2. Study Samples*

Sixty-eight serum samples of DENV-2 confirmed cases from the Brazilian states of Rio de Janeiro, São Paulo, and Minas Gerais, collected between 2007 and 2019 were included in our study. Samples were sent to our laboratory by spontaneous demand for diagnostic purposes, accompanied by their respective epidemiological sheets but with patients' identification already encoded. The cases analyzed here were clinically classified according to the 2009 World Health Organization guidelines [2].

### *2.3. Immune Response Classification*

An in-house ELISA assay was employed to titer anti-DENV-2 IgGs. IgG titer was correlated with patients' days of symptoms to determine the primary or secondary character of the immune response against DENV (See Table S7), as described by Miagostovich et al. in 1999 [21].

### *2.4. Viral RNA Isolation and Quantification*

Total RNA was extracted from 140 µL human sera samples employing the QIAmp Viral RNA Mini kit (Qiagen, Hilden, Germany), under the manufacturer's instructions. Extracted RNA (5 µL) was quantified by real-time RT-PCR on an AriaMx Thermal cycler (Agilent Technologies, Santa Clara, CA, USA), following protocols described by Santiago et al. in 2013 [22]. For the RNA quantification, a standard calibration curve was constructed using serial dilutions of RNA extracted from the DENV-2 strain 40247 [23], with an initial concentration of $5.01 \times 10^5$ pfu (plaque-forming unit)/mL.

### *2.5. Virus Genome Deep Sequencing*

An initial clarification step was performed for samples 160–209 by centrifuging sera at 3000× *g* over 30 min at 5 °C. Subsequently, the supernatants were passed through 0.22 µm filters to remove bacterial cell-sized particles and other particulate debris. An aliquot of 140 µL of these filtered sera, plus remaining serum samples (137–159) was supplemented next with 0.1 M β-mercaptoethanol, and total RNA was extracted as described in the previous section, with the exception that the final elution was carried out with 20 µg/mL Linear Acrylamide in RNase-free water ($H_2O$-LA). Extracted nucleic acids (60 µL) were immediately treated with four units of Turbo DNase (Life Technologies, Carlsbad, CA, USA) to digest DNA contaminants, and were purified with Agencourt RNA XP beads (Beckman Coulter Genomics, Chaska, MI, USA), with a final elution in 30 µL of H2O-LA.

Selective depletion of human ribosomal (rRNA) and carrier RNA from viral RNA samples, randomly-primed cDNA synthesis, and library construction were performed, as described previously [24], with slight differences for samples 160–209: (i) the dnased-RNA samples were treated with a non-thermostable RNase H (New England Biolabs, Ipswich, MA, USA) for selective contaminant RNA depletion, employing the commercial enzyme buffer and performing the reaction at 37 °C instead of 45 °C; (ii) cDNA synthesis was performed using the Superscript-IV reverse transcriptase system (Invitrogen), reducing the first-strand synthesis reaction time from 50 to 10 min; (iii) libraries were constructed by employing the Illumina DNA Prep kit (formerly named Nextera DNA Flex) with reaction volumes reduced to half the recommended, to reduce tagmentation and number of integration sites. Eighteen cycles of libraries PCR amplification were performed, following the manufacturer's cycling conditions. Finally, the multiplexed libraries' yield was determined with an 'Agilent High Sensitivity DNA kit' in a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and quantified with a Qubit fluorometer (Life

Technologies, Carlsbad, CA, USA). Libraries were pooled in equimolar concentrations and paired-end sequenced in one of the Illumina's system: Nextseq500 (75 cycles; samples 137–159) at the Institut Pasteur, France, or HiSeq4000 (300 cycles; samples 160–209) at the Novogene company, EUA. 1–5% PhiX library was employed in both cases as a control for Illumina sequencing runs.

Two DENV-2 negative human sera were processed and sequenced together with the samples to discard potential cross-contamination among samples. Additionally, two libraries were constructed from synthetic commercial plasmids (pGEM-3Zf, Promega, and pUCIDT-Kan, Integrated DNA Technologies) to assess the potential artefactual errors introduced by libraries PCR amplification and sequencing.

### *2.6. Bioinformatics Data Processing*

Sequencing reads were demultiplexed using bcl2fastq v2.15.0 (Illumina). Paired-end reads from each sample were first trimmed by removing Illumina adapter sequences and bases of low quality using Trimmomatic v0.36, with the following settings: ILLUMINACLIP:NexteraPE-PE.fa:2:30:10:5:true LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:20 [25]. Next, depletion from human contaminants was carried on with BMTagger (ftp://ftp.ncbi.nlm.nih.gov/pub/agarwala/bmtagger/) and BLASTN [26], and the reads were then filtered to DENV-2 serotype using LASTAL [27] and JX669477/JX073928 (GenBank accession numbers) as the reference sequences. The resulting reads were de novo assembled by employing Trinity [28], and the contigs were scaffolded and refined with MUMMER [29] and MUSCLE [30]. The de novo assembled consensus sequence was then employed as a reference where trimmed + filtered reads were mapped on to, using Novoalign (http://www.novocraft.com/products/novoalign/). The above software (except Trimmomatic) was implemented in a publicly available pipeline described by Park et al., 2015 [31] and run under the default settings. To limit the influence of PCR artefacts, duplicated reads were thereafter removed with Picard's MarkDuplicatesWithMateCigar tool (http://broadinstitute.github.io/picard/) (with settings: –REMOVE_DUPLICATES = true –AS = true –SKIP_PAIRS_WITH_NO_MATE_CIGAR = true), and the Genome Analysis Toolkit (GATK, https://software.broadinstitute.org/gatk/) was employed to identify variant positions and realign reads around insertion/deletion (indels) positions (IndelRealigner option). Finally, quality scores were added to indels positions with Lofreq v.2.1.3.1 (option indelqual), and intrahost variants [iSNVs and iLVs (including deletions and insertions)] and their proportion among all DENV-2 sequencing reads were called with the same software, by only considering alternate bases with Phred quality equal to or higher than 30 (-Q 30) [32]. Variant calls with a significant strand bias ($p < 0.05$) and a frequency lower than 0.5% were removed from the dataset obtained for each sample (cut-off value inherently obtained from the variant-calling analysis of both the commercial plasmids and the PhiX sequencing control). Moreover, for samples with a low coverage depth (i.e., <100X), variants passing the above filters but present in only one forward and reverse reads (2 reads total) were also discarded to diminish the errors inherently associated with samples' processing and NGS. A final manual edition on the consensus sequences was carried out by considering the variants passing all filters but presenting an intrahost frequency higher than 50%. Samtools v1.9 [33] was employed as additional software to convert, sort, and index files.

After human sequence depletion and filtering to only keep the DENV-2 sequences, fastq files for all sequences generated in this study were deposited in the NCBI-Sequence Read Archive under the BioProject ID PRJNA541495.

### *2.7. Phylogenetic Analysis*

Whole-genome consensus sequences obtained by deep-sequencing were aligned using Mega v7.0 [34] to 321 DENV-2 sequences available in GenBank (https://www.ncbi.nlm.nih.gov; as retrieved on 10 September 2020, keeping only the full genome sequences (GenBank Accession numbers can be found in Table S8). Sequence alignment was next

manually curated to remove the artefacts. Evidence for potential recombination events in the alignment consensus sequence was discarded (Phi-test; $p = 0.3427$) with the Recombination Detection Program v.4.101 (http://web.cbio.uct.ac.za/~darren/rdp.html). Maximum likelihood phylogenetic trees were constructed using the RaxML v8.2.8 software [35] with 1000 bootstrap replicates, under the GTR + I + G substitution model obtained by Akaike's information criterion and likelihood value in Jmodeltest v2.1.6 [36]. The consensus tree was visualized in FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

### *2.8. Intrahost Viral Genetic Diversity Assessment*

To analyze the existence of patterns of DENV-2 intrahost diversity in samples, according to patient's clinical classification, the iSNV/LVs profile obtained after variant calling was first assessed for each sample. Since the variant-calling procedure returned each minor variant's position in the complete viral genome (nucleotides 1 to 10723), their specific position on each particular viral gene was computed by employing an in-house script developed in R v3.5.3 (https://www.r-project.org/). Next, the impact of iSNV/LVs on each viral gene and amino acid encoding protein was obtained with the SnpEff software v4.3 [37]. iSNV/LVs identity, frequency, position, and impact over viral protein were then assessed for each clinical classification category, normalizing by group size to avoid biasing. The same procedure was carried on for the cases clustered by the immune response.

### *2.9. Natural Selection Assessment*

The magnitude and direction of intrahost natural selection were estimated with the ratio of non-synonymous (dN) to synonymous (dS) substitutions per non-synonymous and synonymous coding sequence sites, using the Jukes-Cantor formula, as described elsewhere [38]. The dN/dS ratio represents a measure of selective pressure; thus, a ratio dN/dS > 1 results when changes in the protein sequence are favored by natural selection (evidence of positive selection), while a ratio < 1 is expected if natural selection suppresses protein changes (evidence of negative selection). A dN/dS ratio equal to 1 represents a situation of neutral evolution [38]. The DnaSP software v6.12 [39] was employed to obtain the number of synonymous and non-synonymous sites for each sample consensus sequence.

### *2.10. Statistical Analysis*

Statistical comparisons were performed using the GraphPad Prism v7.0 (https://www.graphpad.com/scientific-software/prism/).

## 3. Results

### *3.1. Samples Characteristics*

Sixty-eight DENV-2 cases were clinically classified according to the WHO Guidelines [2] as follows—(a) dengue fever (DF) (45.6% of total cases, $n = 31$), (b) dengue with warning signs (WS) (27.9% of total cases, $n = 19$), and (c) severe dengue (SD) (26.5% of total cases, $n = 18$). Two and twelve WS and SD cases, respectively, were identified as fatal cases. Cases presenting a primary infection were 57.4% ($n = 39$), while the remaining 42.6% ($n = 29$) presented a secondary infection. When comparing the patient's viral load at the time the samples were collected, the DF cases presented a higher median concentration (Table 1; Kruskal-Wallis test, $p < 0.0001$). When cases were grouped by the days since the onset of symptoms, viral load median also resulted higher for samples taken up to two days since the onset of symptoms, as expected, compared to those with three days or more (Kruskal-Wallis test, $p = 0.0002$). Indeed, in this cohort, clinical classification correlated strongly with patients' days of symptoms (Pearson r correlation, $p < 0.0001$; Table S1), consistently with previous findings [40]. On the other hand, however, no statistic-supported association was detected between clinical classification and patients' immune response (Table S1). As well, viral load medians did not differ statistically among cases with a primary or secondary immune response (median [interquartile range (IQR)]: $2.61 \times 10^4$ [$1.06 \times 10^2$–$5.17 \times 10^4$]

and $7 \times 10^3$ [$2.36 \times 10^2$–$8.24 \times 10^4$] pfu/mL, respectively) (Mann-Whitney test, $p > 0.05$). The samples' epidemiological and laboratory data are summarized in Table 1 (See Table S2 for detailed depiction).

**Table 1.** Epidemiological information of DENV-2 samples.

| Clinical Classification | Number of Cases [*n* (%)] | Gender [M: F (%)] | Age [Mean (Min–Max )] | Location [State (%)] | VL (pfu/mL) [Median (Min–Max )] | Days of Symptoms [Mean (Min–Max)] | Immune Response [*n* (%)] |
|---|---|---|---|---|---|---|---|
| DF | 31 (45.6%) | 15:16 (48.4–51.6%) | 39.2 (10–65) | RJ (51.6); MG (48.4) | $4.31 \times 10^4$ ($2.47 \times 10^2$–$8.38 \times 10^6$) | 2.2 (0–5) | *p* = 17 (54.8%) S = 14 (45.2%) |
| WS | 18 (27.9%) | 12:7 (63.2–36.8%) | 36.7 (8–85) | RJ (84.2); MG (10.5); SP (5.3) | $3.78 \times 10^3$ ($4.68$–$1.32 \times 10^6$) | 2.9 (0–12) *a | *p* = 8 (42.1%) S = 11 (57.9%) |
| SD | 17 (26.5%) | 6:12 (33.3–66.7%) | 31.7 (7m-88) *a | RJ (77.3); SP (22.2) | $1.48 \times 10^2$ ($2.86$–$4.94 \times 10^5$) | 7.1 (4–24) *b | *p* = 14 (77.8%) S = 4 (22.2%) |
| Total | 68 | 33:35 (48.5–51.5%) | 36.6 (7m-88) | RJ (67.6); MG (25.0); SP (7.4) | $1.21 \times 10^4$ ($2.86$–$8.38 \times 10^6$) | 3.6 (0–24) * | *p* = 39 (57.4%) S = 29 (42.6%) |

DF: dengue fever; WS: dengue with warning signs; SD: severe dengue; M: male; F: female; RJ: state of Rio de Janeiro; SP: state of São Paulo; MG: state of Minas Gerais; VL: viral load; pfu: plaque formation unit; P: primary infection; S: secondary infection; * a: information not determined for one sample; and * b: information not determined for two different samples.

### *3.2. Phylogenetic Analysis*

After samples' deep-sequencing, a full-length viral consensus sequence was obtained for each sample, which were included in a dataset composed of 321 DENV-2 complete genomes retrieved from GenBank. Next, the aligned sequences were phylogenetically analyzed. As expected, all of this cohort's specimens corresponded to genotype III (formerly known as Asian/American), which is circulating in Brazil since its introduction in 1988–1989. However, samples grouped within two different clusters with 100% bootstrap supported (Figure 1) Brazilian lineage BR3, which corresponded to viral strains circulating after DENV-2 re-introduction in the state of Rio de Janeiro in 2007, and the newly described lineage BR4, which included strains circulating in the country since 2016 [41,42]. Even though BR4 might be a new lineage spreading through Brazil, their representing sequences still grouped within the clade IV of genotype III and was not as genetically diverse from BR3. Additionally, contrary to what was described for BR1 and BR3 [16], no differences were observed in the clinical–epidemiological pattern between BR3 and BR4. Thus, isolates belonging to BR4 lineage were not excluded from this study.

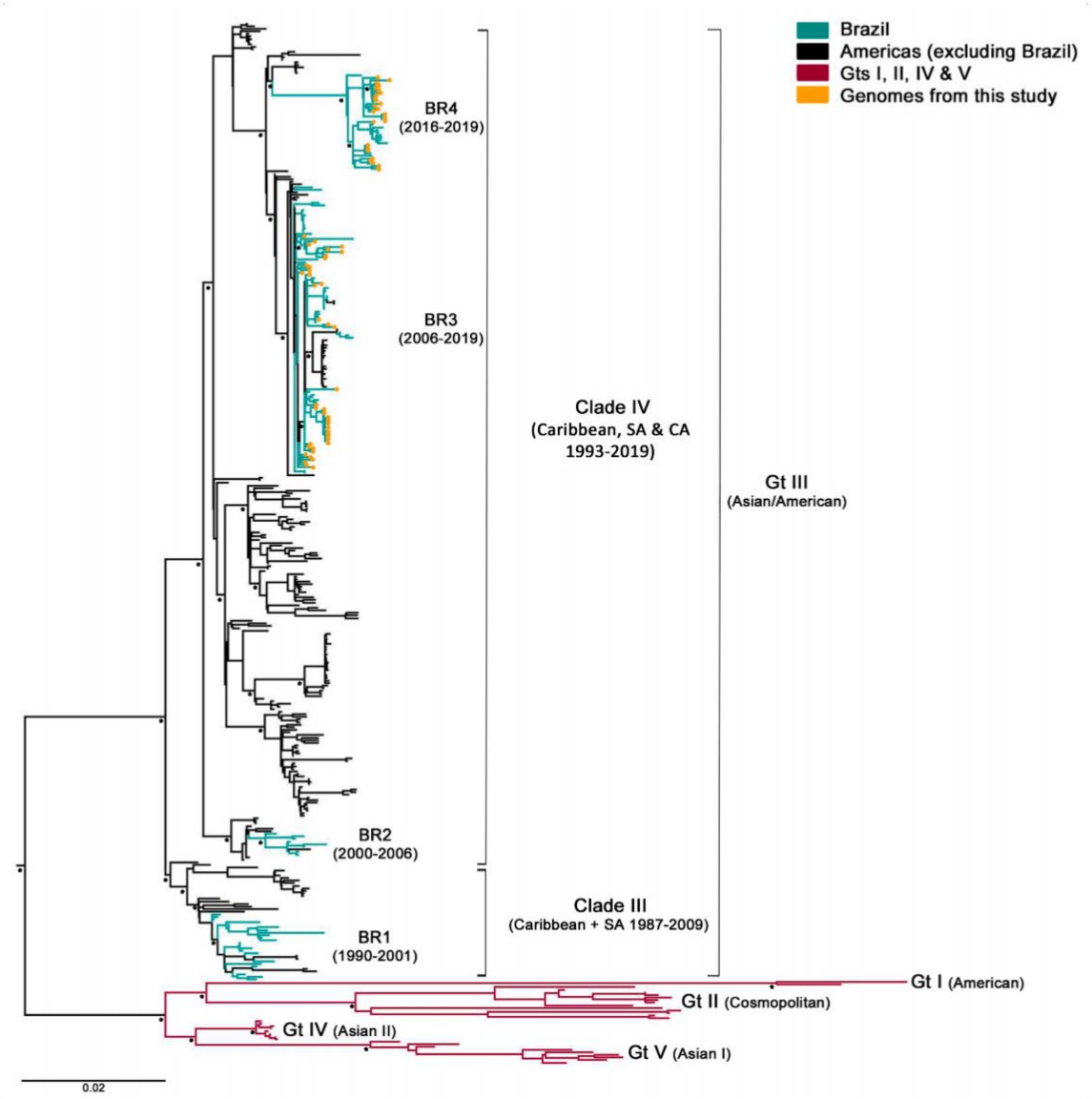


**Figure 1.** Maximum likelihood phylogenetic tree of DENV-2 polyprotein. It was constructed in RaxML v8.2.8 under GTR + I+G substitution model (General Time Reversible with gamma distribution and invariant sites), and 1000 bootstrap replicates. Main nodes with more than 70% of replicate trees of the bootstrap test for which the taxa clustered together are denoted with a black star. Brazilian sequences obtained in this study are represented in the tree with an orange circle. Gt: genotype; SA: South America; CA: Central America; and BR1-4: Brazilian lineages 1 to 4.

### *3.3. Intrahost Viral Population Structure*

DENV-2 genome was next analyzed in-depth, to look for any particular pattern among clinically clustered samples. The median DENV-2 genomic coverage depth was 465.5 (range 8.75–6030) (Table S2). To limit the potential biases introduced by coverage depth variation, ultra-rare variants with frequencies lower than 0.5% were discarded, as well as any presenting considerable strand-bias.

After mapping sequencing reads of each sample to its corresponding consensus sequence and proceeding with the variant-calling, a total of 9660 intrahost single nucleotide variants (iSNV) and 520 length variants (iLV, representing insertions or deletions) were detected among all samples (see Table S3). To analyze the existence of intrahost diversity patterns, iSNVs and iLVs generated during virus replication were ordered along the viral

genome. Then, each gene's total number was computed for each sample and normalized to the gene's length to obtain its variability (percent of variations per total nucleotide positions). Finally, median variability for each gene was plotted for the clinically clustered samples (Figure 2A). The same procedure was carried out for the cases that were clustered by immune response (Figure 2D). Even though no correlation seemed to link clinical outcome with immune response in this sampling, it is known that biologically, the immune response is an important factor that might contribute to the severity of the disease [3]. Thus, analysis with samples grouped by this characteristic was performed either way. Overall variability along the complete viral genome was not significantly different between any three clinical groups (Kruskal–Wallis test, $p > 0.05$). However, it did differ notably between the genome regions for SD cases (Kruskal–Wallis test, $p = 0.0002$), with NS2A, NS4A, and NS4B presenting the highest values. Particularly, NS2A and NS2B median variability were higher than that observed for DF (median [IQR]: 2.82% [0.58–3.73] vs. 0.8% [0.3–2.4], $p = 0.0301$ for NS2A, and 1.8% [0.6–2.8] vs. 0.5% [0–1.5], $p = 0.0259$ for NS2B; Mann–Whitney tests). Additionally, NS4A median variability results were also higher than that of WS cases (3.45% [0.85–3.85] vs. 0.4% [0.2–3.1]; Mann–Whitney test, $p = 0.0471$) (Figure 2A).

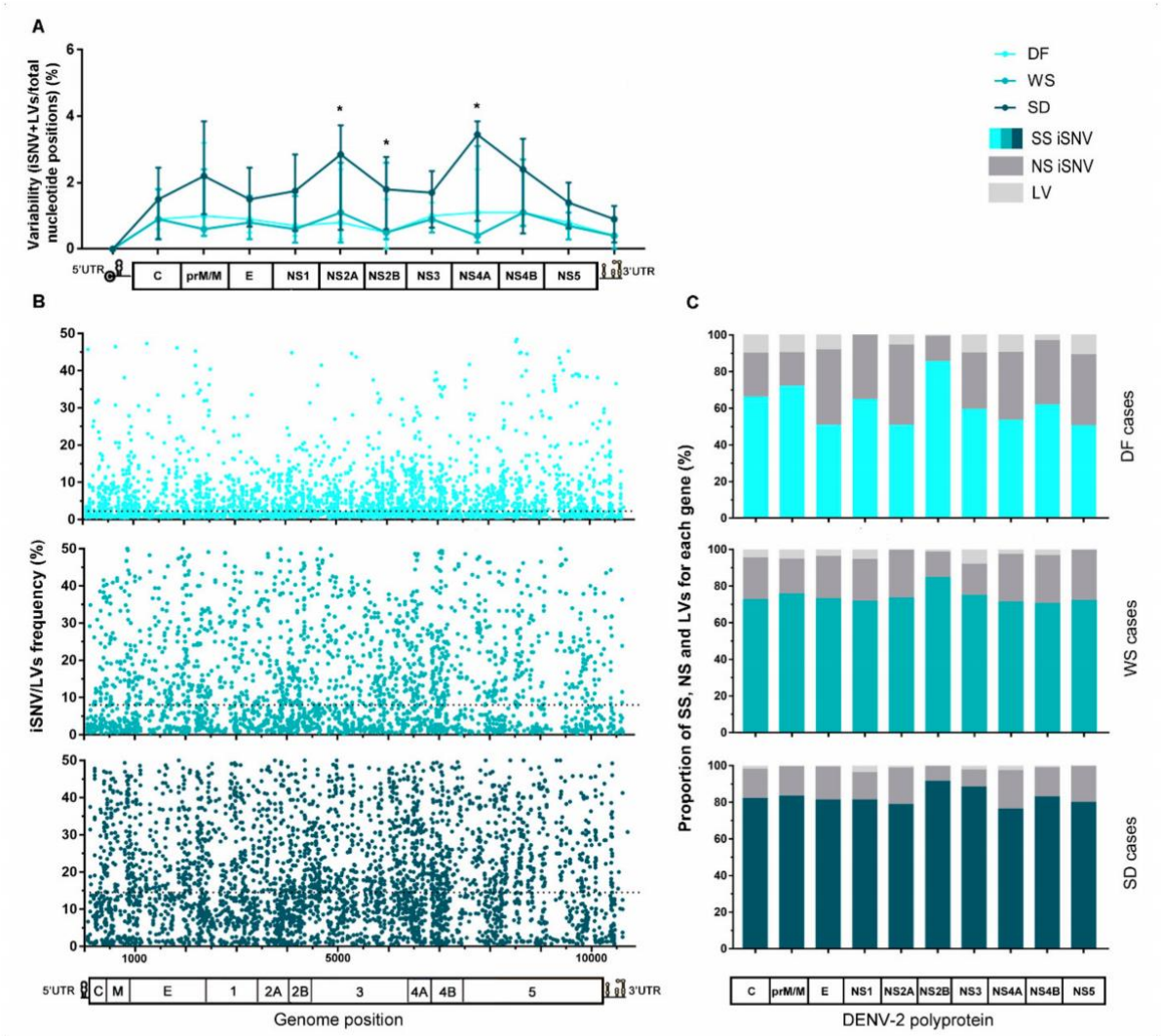


**Figure 2.** *Cont.*

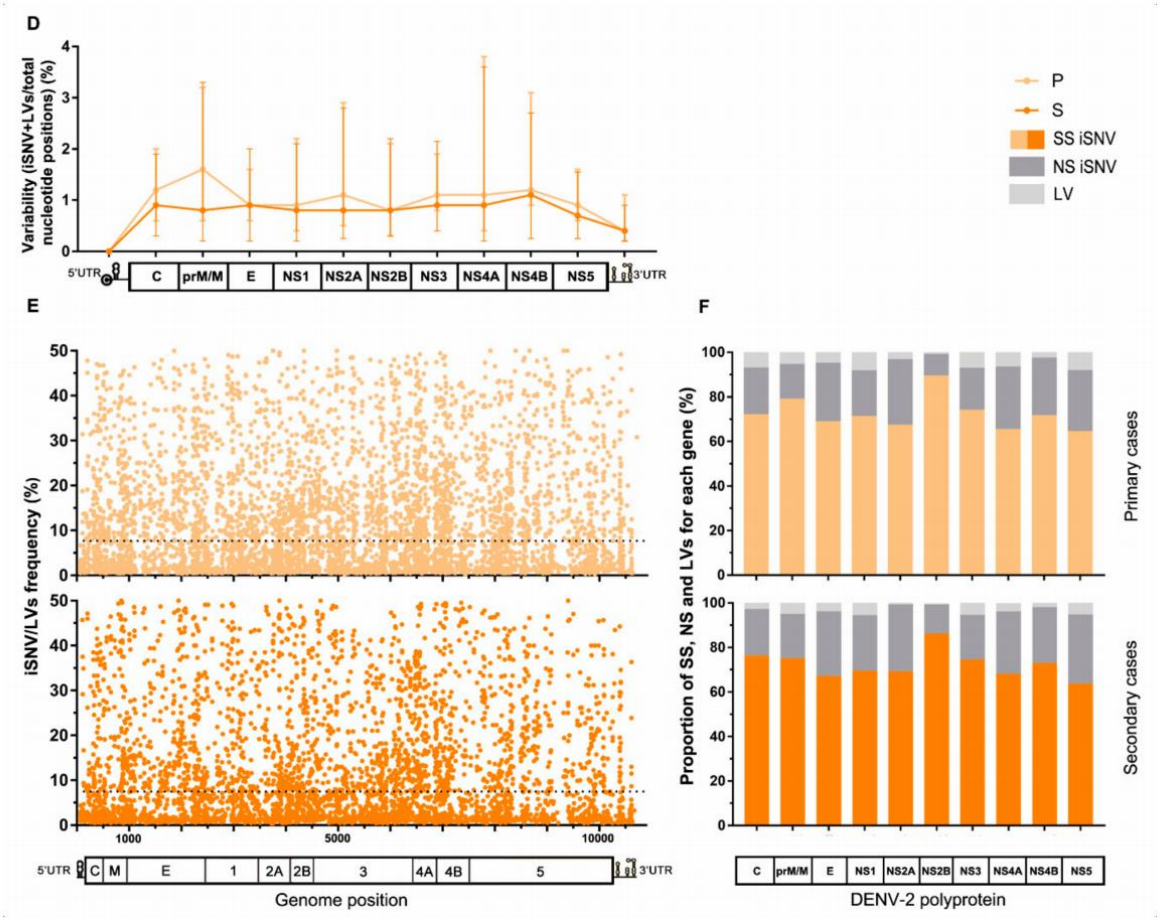


**Figure 2.** Characteristics of the intrahost viral population of cases grouped by clinical category or immune response. (**A**) Variability along the viral genome according to the patients' clinical outcome. The total number of iSNVs located in each gene along the viral genome was normalized by each gene's length (total nucleotide positions) and the median value for each group plotted in the graph, with error bars representing the interquartile ranges. (*) Statistically-supported differences among clinical groups. (**B**) iSNV/LVs frequency distribution along the viral genome according to the clinical category. The dotted line represents the median frequency among all iSNV/LVs found within each group. (**C**) Percentage of synonymous, non-synonymous iSNVs, and LVs for each gene. Clinical categories are represented separately. The total amount of each variant class per gene was summed and then normalized by group size. (**D**–**F**) The same analysis was performed for cases grouped by patients' immune response. Genome/polyprotein schemes in graphs A, C, D, and F are not scaled to genes real size, as are the schemes in graphs B and E. DF: dengue fever cases, WS: dengue with warning signs, SD: severe dengue cases, P: primary cases, S: secondary cases, SS: synonymous variants, NS: Non-synonymous variants, and LV: insertion/deletion variants.

The intrahost frequency of every SNV/LVs within each host's viral population was determined during the variant-calling process. The median of the frequency was calculated for all iSNV/LV detected in the total number of samples compounding each category. The lower frequency obtained for the DF cases suggests that viral subpopulations are less represented (median [IQR]: 2.25 [1.16–6.30], while for WS and SD it was 8.00 [2.21–20.00] and 14.49 [7.32–26.50], respectively. Kruskal–Wallis test, $p < 0.0001$). All iSNV/LVs were also combined by genome position and clinical classification (Figure 2B) or immune status (Figure 2E) to assess their frequency distribution along the genome. It was clearly shown that variants within the DF cases were present at minor frequencies indistinctive of genome position (Figure 2B). Furthermore, by observing the effect of each iSNV/LVs over the genome coding region, it was observed that DF presented an overall higher proportion of non-synonymous iSNV (NS-iSNV) and iLVs ($\bar{x} \pm$ Sd: 31.75 $\pm$ 9.99 and 8.2 $\pm$ 3.98) than WS (22.6 $\pm$ 4.6 and 4.41 $\pm$ 2.76) and SD (15.9 $\pm$ 4.3 and 1.63 $\pm$ 0.98) (one-way ANOVA test;

$p = 0.0011$ and $p < 0.0001$ for NS-iSNV and LV, respectively) (Figure 2C). This observation should be taken together with the shorter infection time of the DF cases and the possibility of many of these variants being deleterious or detrimental to viral fitness. The proportion of synonymous iSNVs (SS-iSNV) for DF cases, which was consequently lower for WS and SD (1-way ANOVA test; $p = 0.0011$) also presented more fluctuation within this group (range: 50.4–85.5), when compared to the other two (70.7–84.7 and 76.4–91.6 for WS and SD, respectively) (Figure 2C). Particularly, E, NS2A, NS4A, and NS5 were the genes that presented the lower proportion of SS-iSNVs (50%); a value that raised to 71–73% and 76–81% within WS and SD, respectively. It is worth noticing that the NS2B gene presented the highest proportion of SS-iSNVs among all coding-genes, irrespective of the patient's clinical outcome (85% for DF and WS; 92% for SD), which could be likely related to a low tolerance to mutations in the translated protein.

On the other hand, for samples grouped by patient's immune response, a similar variability pattern was observed along the entire genome irrespective of the immune response, with no considerable difference for any particular genome region (Mann–Whitney tests, $p > 0.05$) (Figure 2D). As well, no substantial difference was found between the iSNV/LVs' frequency medians of both groups (median [IQR]: 7.7 [1.94–18.75] for primary, and 7.5 [2.01–17.94] for secondary cases; Mann–Whitney test, $p > 0.05$) (dotted line, Figure 2E), nor in the comparison of the medians for each particular genome region, except for NS2B, where the primary cases presented a discrete higher median frequency (median [IQR]: 10.64 [4.35–19.12] vs. 7.0 [2.83–16.9] for secondary cases; Mann–Whitney test, $p = 0.0183$). Next, when checking the effect of the iSNVs on the coding genes, slightly higher proportions of NS-iSNVs were noticed among the secondary cases for prM/N and NS1 in the first place, followed by NS2B and NS5 (Paired *t*-test; $p = 0.0132$). On the contrary, primary cases showed slightly higher proportions of iLVs in C and NS1 first, followed by NS4A and NS5 (Paired *t*-test; $p = 0.0022$) (Figure 2F). It is interesting to note that C, prM/M, and NS1 are the viral genes on which the immune system's pressure causes the greatest impact [43–45].

Based on the above pieces of evidence, a subsequent detailed analysis of all iSNV/LVs detected in this sampling was carried out. It was observed that from the whole dataset comprising 10,180 intrahost variants, 8948 accounted for 1474 different variants that were consistently repeated among samples, leaving a remaining of 1232 unique variants. After grouping data by clinical outcome, no significant difference was detected for the proportion of unique variants among each group's samples (one-way ANOVA test, $p > 0.05$; Figure S4). Thus, to look for any mutational pattern that could be correlated with the disease's clinical outcome, special attention was paid to repetitive NS-iSNVs (repNS-iSNVs), hypothesizing that if minor variants with the potential to alter viral fitness were transmitted, then they could be identified from repetitive patterns among samples. In this regard, and considering that numerous alterations in the UTR regions were indirectly linked to pathogenesis [46], iSNVs located in those regions were added to the repNS-iSNVs repertoire for exhaustive analysis. Variants composing this subset (344 different NS-iSNV + 41 iSNVs located in the UTRs) were grouped according to their genome position and to their clinical group, normalized by group size, and finally plotted on heat maps (Figure 3). A similar analysis was performed on samples classified by the patient's immune status to try to address whether repNS-iSNVs could arise as a result of immunological pressure (Figure 3).

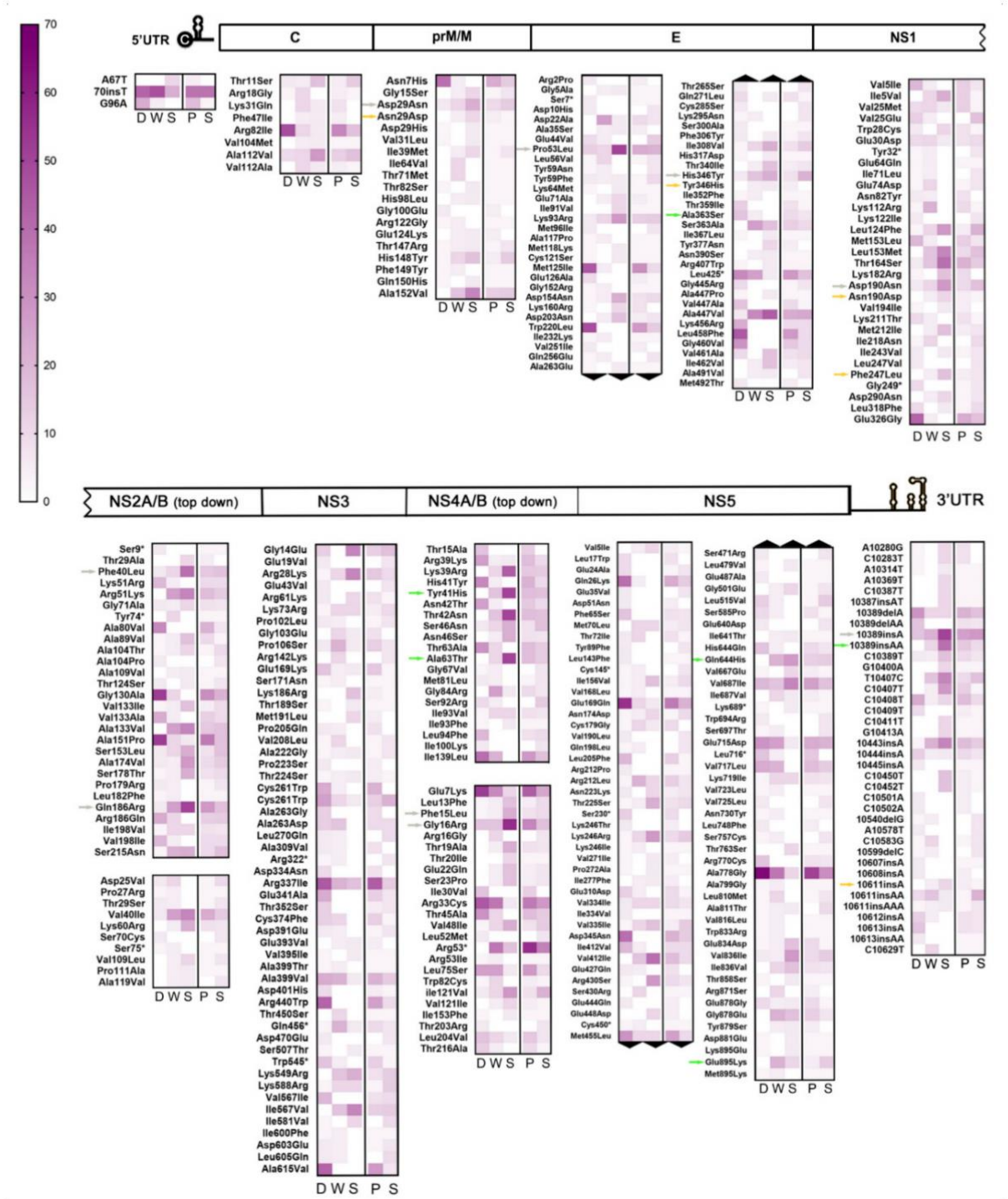


**Figure 3.** Interhost frequency of iSNV/LVs consistently detected among samples. On this graph, only those repNS-iSNV + UTR-iSNVs found in at least two different samples were considered for analysis. From left to right, F: DF cases, W: WS cases, S: SD cases; *p*: primary infection, and S: secondary infection. Colored-scale indicates the percentage of positive samples, with color intensity and numeric scale increasing with interhost frequency. Colored arrows indicate the most relevant variants within subgroups 2 (green), 3 (orange), and 4 (grey).

Interestingly, 65 repNS-iSNVs + 10 UTR-iSNVs were exclusively found among DF cases (subgroup 1), while 77 repNS-iSNVs + 14 UTR-iSNVs were present only among WS and SD cases (subgroup 2). Of these, 17 repNS-iSNVs + 2 UTR-iSNVs (DF), and

23 repNS-iSNVs + 1 UTR-iSNVs (WS + SD) were highly represented among all samples ($n \geq 5$). To highlight, 10/17 and 9/23 of the latter represented variants with non-conservative amino acid substitutions (aa), i.e., residues whose physicochemical properties would be altered (Table S5). However, it is also important to mention that intrahost frequency was considerably low for repetitive variants amid DF cases (median: 1%, range: 1–34%), suggesting that they might not be influential for their respective mutant swarms. In the case of subgroup 2, on the contrary, 5 of the repNS-iSNVs with relevant aa substitutions + 1 UTR-iSNV were present amid the WS and SD cases in high intrahost frequencies (median: 21%, range: 1–47%). They are denoted in Figure 3 with green arrows (for further information, see Table S5). In addition, none of these highly represented variants was found exclusively among primary or secondary cases.

On the other hand, another subgroup of 46 repNS-iSNVs + 10 UTR-iSNVs was also found to be exclusive amid the WS and SD cases (subgroup 3), however, with a lower interhost frequency ($n$ = 2–4). This time, the 24/46 variants carried a non-conservative aa substitution (Table S5), and 12 of them were present only amid the primary cases. Additionally, 4 of the repNS-iSNVs with relevant aa substitutions + 1 UTR-iSNV were present in high intrahost frequencies (median: 24%, range: 3–41%). These are highlighted in Figure 3 with orange arrows.

Finally, a fourth subgroup composed of 58 repNS-iSNVs + 8 UTR-iSNVs stood out from the repetitive variants' subset because they were found in a high interhost frequency ($n$ = 5–19) and in the three clinical categories, but with a marked tendency of increasing/decreasing interhost frequency from the DF to WS + SD cases. Particularly, 22 were highly represented among the DF cases (10–71% vs. 5–37% and 0–17% for WS and SD, respectively), with 5 being located in the UTRs and 11 carrying non-conservative amino acid substitutions. Simultaneously, the remaining 44 were mostly present amid the WS + SD cases (5–32% WS + 11–56% SD vs. 3–19% DF), with 3 located in the UTRs and 15 carrying non-conservative aa substitutions (Table S5). Among the latter, 8 repNS-iSNVs with relevant aa substitutions + 1 UTR-iSNV were found at high intrahost frequencies (median: 16%, range: 1–49%) (Figure 3, grey arrows). This fourth subgroup drew attention because even though these variants did not belong to any particular clinical group, the differences between their interhost frequencies were evident enough. It is also known that depending on the surrounding variants within their respective mutant swarms, they could become relevant [47].

The relevant question here is whether any or all of the above-described variants do actually ascribe the viral particle with more harmful properties, and ultimately whether the improved viral fitness can be correlated to patients' clinical outcome. However, it was observed that despite the fact they were detected at high inter and intrahost frequency and led to potential relevant residues substitutions in the respective proteins, or changes in the UTRs, for 70% of them (14/20), the inverse iSNV was detected in the other DF cases. For example, while 4 WS + SD cases carried the T3162A-Phe247Leu iSNV in NS1, another DF case carried the A3162T-Leu247Phe iSNV. This means that actually, despite the Leu247 variant being present at a high proportion within the WS + SD viral populations, it was detected at the consensus level (represents the majority of the viral population; allele frequency higher than 50%) in a DF case, which ultimately decreased the chance of that particular variant being associated with a severe clinical outcome. A new approach and further studies would be needed to determine their effect on the viral populations.

### *3.4. Intrahost Selective Pressure*

Natural selection acting on viral populations was assessed by calculating the ratio of non-synonymous (dN) to synonymous (dS) substitutions per coding sequence site (dN/dS), considering both iSNVs and iLVs. Almost all samples ($n$ = 61/68; 89.7%) presented dN/dS ratios <1, representing potential evidence that a purifying selection pressure might be taking place to shape the viral populations under study. However, SD cases exhibited dN/dS ratios lower than the DF cases (Mann–Whitney test DF vs. SD; $p < 0.0001$), suggesting that

DENV-2 was under a stronger purifying selection in the SD cases (Figure 4), in line with their longer infection times. This is true also for dN and dS when analyzed separately, suggesting that the differences observed between the DF and SD cases were both due to differences in the rates of substitutions at the silent (dS) and non-silent (dN) sites (see Figure S6). Based on the hypothesis that under a prolonged exposure of the virus to the host immune system, only some subpopulations would be positively selected for, we grouped samples by days of symptoms and observed that actually, the median for the dN/dS ratio was the highest for cases with 1 day since the onset of symptoms, with a concomitant decrease up to day 5, correlating with an increased exposure time to the purifying selection (see Figure S6).

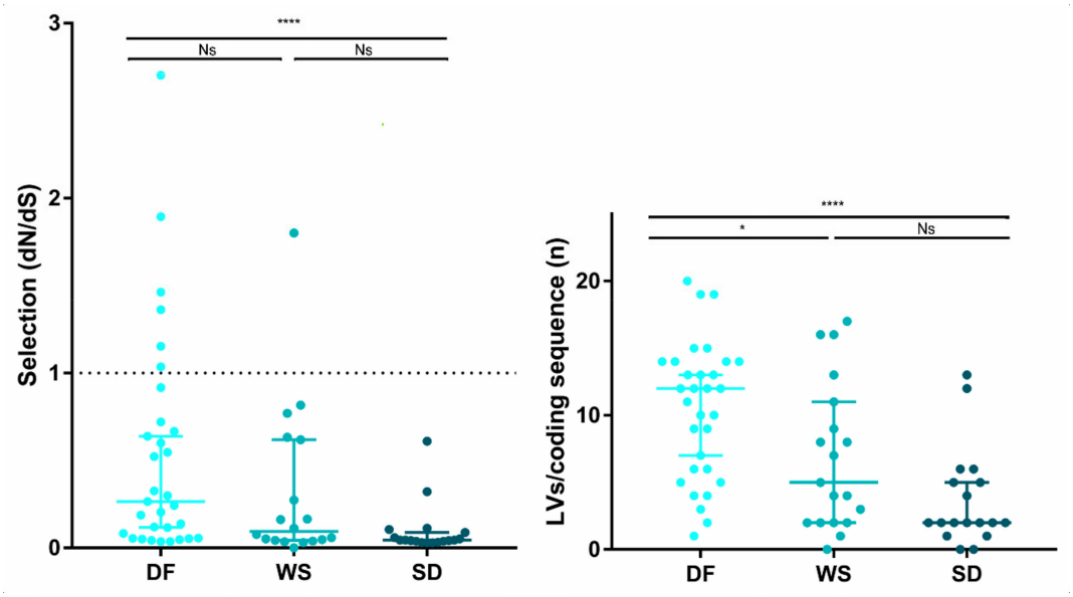


**Figure 4.** Natural selection strength assessment. The strength of host selection on virus populations was compared between clinical categories. Left: dN/dS ratio over all coding positions. dN/dS ratio of 1, is interpreted as evidence for neutral evolution (dotted line). dN/dS > 1 represents positive selection, while dN/dS < 1 represents a negative (purifying) selection. Right: Number of accumulated iLVs. In all cases, each dot represents a sample and the lines are the median with the IQR. (*) $p < 0.01$; (****) $p < 0.0001$; Ns: not significant.

Deepening the analysis to check whether the different genes were subjected to a distinct natural selection strength, the dN/dS ratio was also computed for each coding gene within each sample and then analyzed for the samples grouped by clinical classification, immune response, or days of symptoms (see Figure S6). Purifying selection was the strongest in the NS2B and NS3 genes, irrespective of clinical or immune response classification, and days of symptoms. NS2A seemed conspicuously subjected to a stronger negative selection in the WS and SD cases than in the DF cases (Mann–Whitney test, DF vs. WS $p = 0.0346$; DF vs. SD $p = 0.0134$), which was in line with the higher proportion of non-synonymous substitutions found for this latter group. Additionally, a non-statistically supported slight difference was observed in this gene between samples with less than 3 or more than 4 days of symptoms, indicating that selective pressure over this gene might appear later during the infection process. On the other hand, the purifying selection result was weaker for the NS4A gene, which presented the highest dN/dS ratios, independent of the sample classification. Interestingly, and opposite to what was expected, no higher ratios for the highly-immunogenic C, prM/M, E and NS1 coding genes were observed [43–45,48]. However, one caveat about these findings, was that the dN/dS variation along the polyprotein could be assessed only for 32/68 samples. For the remaining, the absence of iSNVs in particular genes led to an unviable mathematical calculation of the ratio for that gene, thus,

nullifying the analysis for the whole polyprotein. Due to this potential limitation, these last results should be considered cautiously.

Grubaugh et al. proposed that iLVs in the coding sequences are predicted to be deleterious and, consequently, rapidly removed by natural selection [49]. In this context, the strength of the purifying selection was also assessed by analyzing the number of accumulated iLVs per coding sequence (LV/cseq) within the samples grouped by clinical outcome. The SD cases showed the lowest median LV/cseq (median [IQR]: 2 [1.75–5.25]) followed by the WS (5 [2–11]) and the DF cases (12 [6–14]) (Kruskal–Wallis test, $p = 0.001$) (Figure 4).

## 4. Discussion

Several investigations showed evidence of a strong association between the genetic composition of intrahost viral populations and viral fitness and pathogenicity [9–12], especially lately that high-coverage and accurate whole-genome sequencing provided proof that fitness and adaptability variations occur without changes in the viral consensus sequence [19,20,50]. However, as recently reviewed by Ko et al., few studies tracked intrahost DENV diversity under epidemiological settings [19,20,50,51]. All of them combined viral genome PCR amplification + deep sequencing experimental strategies. Unlike them, this study employed an amplicon-free in-depth sequencing approach, aiming to reliably reflect the viral genetic diversity at each stage of patient infection, to ultimately assess a correlation with disease severity. To keep that reliability and obtain the patient's snapshots to analyze DENV-2 intrahost diversity meant resigning to high sequencing coverage depth for samples with low viral load. SD cases, which were shown to present overall lower viral loads, were the critical analytical point of this study. Normalizing the initial RNA load to the limiting sample would lead to an important loss of information in the remaining samples. Thus, with the focus of representing the real DENV-2 intrahost scenario within each patient, and the implementation of several filters after variant-calling to reduce potential error-biasing, we performed the analysis with all samples included in the study (despite the depth variation, due to the initial differences in sample viral load), to look for any particular pattern that could arise from the clinically clustered samples, afterwards.

In RNA viruses, the generation of variability is determined by the nature of the error-prone replicative RNA polymerase and its replicative rate, while the prevalence of individual mutants depend solely on the fitness of that particular mutant [47]. In our study, we observed that DF cases presented higher proportions of NS-iSNV + iLVs than WS and SD, thus increasing the chance of detrimental non-synonymous mutations, truncated proteins, and defective viral particles (Figure 2C). Two possible explanations are worthy of consideration—(1) in this highly dynamic genetic state of DF cases, the progression of the disease into severity might not be facilitated, or (2) as suggested by Gregory and collaborators the richer viral genetic diversity present in the DF hosts could be beneficial as it would endow the virus population with a greater capacity to adapt to environmental changes as the disease took its course [52]. However, also considering the overall lower intrahost frequencies at which these variants were found (Figure 2B), and the fact these cases represented infection evolutionary times shorter than WS and SD, the most likely explanation would be that differences observed among the clinical groups might be due to distinct periods of intrahost evolution. The process through which intrahost immune pressure shapes DENV-3 diversity during human infections and how this process can be affected by the human host's immunological background was recently demonstrated [20,50]. Consistently, this would explain why SD cases, submitted to a prolonged and exhaustive purifying selection process (samples taken from day 4 since the onset of symptoms, onwards), accumulated a higher proportion of SS-iSNVs (Figure 2C).

When analyzing how variability distributed along the viral genome, it stood out that despite the slight differences noticed for three non-structural genes among the clinical categories (NS2A, NS2B, and NS4A), a common trend was stressed for the NS2B gene, regarding its low proportion of NS-iSNVs irrespective of patient's clinical outcome (Figure

2C). As mentioned before, this can be construed as an activity of the mutated protein poorly supported by the overall viral population, in which case, the NS-iSNVs detected would be expected to be conservative, or variants of recent evolution, which did not yet get the time to be selected for or against. NS2B is a cofactor of the NS3 protease, necessary for the 7-step catalytic processing of the polyprotein. For a full enzymatic activity, NS3 requires the interaction with the NS2B structural loop to comprise residues 49 to 95 [53]. Parameswaran et al. also observed the lowest intrahost diversity along the viral genome to be in this gene [19]. Taken together, this gene could be considered an exciting candidate or target for drugs/vaccine design.

Contrary to the observations from DENV-3 secondary infections [20], we did not find differences in the overall variability between this cohort's primary and secondary cases. However, secondary infections showed a higher proportion of NS-iSNVs than primary ones, consistent with their shorter infection times (62% presented less than 3 days of symptoms, compared to 38.5% among the primary cases), and likely, with the immune-driven responses. On secondary infections, the heterologous antibodies produced during primary infections could be acting once the virus entered the host, rapidly depurating defective particle product of insertion/deletion variants on the viral genome, which would be consistent with the higher proportion of iLVs found among the primary cases. Additionally, their imposed selective pressure could be the cause of the discrepancies observed for the proportion of NS-iSNV in prM/M and NS1, two highly immunogenic coding genes. To notice, unlike other studies, no distinction could be made between the intrahost diversity of the E gene in the primary and secondary cases [19,20,50].

On the other side, if the heterologous antibodies facilitated virus replication, as the ADE theory claimed, and thus, genetic diversity, it would be expected for secondary cases to present a higher viral load compared to primary ones. Actually, no meaningful difference was detected among their medians. Even though it might seem contrary to common findings in which secondary cases tend to develop severe clinical conditions due to ADE, it is important to consider that Katzelnick et al. described a risky window of pre-existing heterologous antibody titer correlated with increased infection severity [3]. It could be likely that some secondary cases of this cohort had pre-existing antibody titers outside the window, and therefore, eluded severe infection. Indeed, only four of the secondary cases developed SD, and in line with this, three of them presented the highest antibody titers obtained by an in-house ELISA assay (Table S7). Either way, conclusions drawn from the analysis of the cases grouped by primary/secondary immune response should be careful, since no statistical correlation was detected in this cohort between patients' immune response and clinical outcome.

NS-iSNVs + UTR-iSNVs detected amid SD cases could be thought of as the mutants that best adapted to the host environment and the subsequent selective pressure during the 4 or more days of the disease incubation. The UTRs present highly structured domains that mediate viral replication and assist the virus in evading the host innate immune response, hence contributing to determine the infection outcome in the host [reviewed in Reference 46]. Being such constrained areas, it was critical to also assess their presence within this cohort's cases. Therefore, and to determine whether there was any characteristic mutational pattern emerging after clustering samples by their clinical outcome, NS-iSNVs + UTRs-iSNVs consistently found among the samples were classified according to their presence within each group (Figure 3). The first subset of repetitive variants was found exclusively amid the DF cases and in low intrahost frequencies (subgroup 1, Table S5) suggesting that these variants might not be involved in the severe pathogenesis of DENV-2. It is unclear whether they could represent a disruptive factor for the virus, inducing low fitness and ultimately viral extinction, but this could be a possible explanation for their low frequency (also due to shorter infection times) and prevention of DF case progression into severity. On subgroups 2 and 3, composed of variants found only within the WS + SD cases, 5 and 4 repNS-iSNVs, respectively, were detected in a first instance as relevant variants potentially correlated with SD, due to their inter and intrahost frequencies, and

their effect on viral proteins. However, their presence at the consensus level in samples other than these carriers (and mainly DF cases) ruled out this previous hypothesis. On the other hand, this observation could represent a hint that some viral subpopulations could be effectively transmitted after overcoming human–mosquito barriers. Supporting our line of evidence, previous studies with several viral models proved the effective transmission of viral subpopulations, demonstrating that the mutant swarms of viral populations possess minority genomes that reflect those that were dominant at earlier phases of their evolution, serving as a molecular reservoir that is able to swiftly react to selective constraints that were previously experienced by the same population [31,54,55]. From this standpoint, the question arises whether it would be conceivable that these variants were just relevant for transmission but not pathogenesis. Two UTR-iSNVs involving 1–2 nucleotide insertions at positions 10389 and 10611 were also detected in these subgroups (colored arrows on Figure 3; Table S5) but not discarded, as in the previous case. However, 10611insA would not compromise any RNA secondary structure, while 10389insAA is located within stem-loop I, an RNA structure responsible for the generation and accumulation of subgenomic flavivirus RNAs, which play essential roles counteracting antiviral responses in mosquito and human cells [56]. Further studies would be needed to determine the possible effect of this iSNV. Finally, of particular interest were the minor variants detected amongst cases belonging to the three clinical groups (subgroup 4, Table S5). They might be thought of as the variability reservoir capable of conquering human–mosquito transmission bottlenecks, which when exposed to strong purifying selection (as in SD cases), can evolve restoring mutant swarms. It could be likely that repetitive variants of this subgroup might be reflecting this scenario. In turn, 5 of their most relevant repNS-iSNVs were detected at consensus level in up to three WS + SD cases.

Though the aim was the repetitive variants, unique ones should not be set aside. While it is impossible to trace a mutational pattern from them, their participation in the pathogenesis, either by their potential or by possible epistatic effects cannot be dismissed. Regarding the latter, it is also worthwhile to investigate whether the combined effect of repNS-iSNVs detected here amid the SD cases could elicit severe phenotypes. Epistatic relationships were recently documented for DENV [57,58].

Whether there is a potential structural or functional significance for the variants reported here in the DENV-2 genome is an open question. The next step would be to better characterize the effects of these mutations on viral replication and protein interaction, to finally understand the phenotypic traits underlying the viral fitness of these DENV-2 populations and its relation with severe dengue disease. On the other hand, even though this study did not mainly focus on the synonymous SNV repertoire due to its nature, special attention needs to be given in the future to this repertoire, as there is much evidence showing they might not necessarily impair neutral changes [59,60].

The results generated in this study showed that the viral intrahost population structure of these samples varied according to the DENV-2 disease severity, as a straight consequence of the infection time, contributing to understanding the viral dynamics of this virus.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/1999-4915/13/2/349/s1, Table S1—Statistical correlations between patient's clinical outcome and immune response or days of symptoms. Table S2—Clinical, epidemiological, and sequencing sample characteristics. Table S3—iSNV/LVs repertoire of each sample. Figure S4—Percentage of unique variants in mutant swarms of cases grouped by clinical classification. Table S5—Repetitive NS-iSNVs + UTR-iSNVs clustered by subgroups according to their interhost frequency within the clinical categories. Figure S6—Natural selection (dN/dS) assessment for samples grouped by patient's immune response and days of symptoms. Table S7—Immune response classification. Table S8—GenBank accession numbers of the DENV-2 sequences employed in the phylogenetic analysis.

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review and editing, M.C.T., A.S. and A.M.B.d.F.; visualization, M.C.T., M.C.L.d.M. and A.M.B.d.F.; supervision, M.C.L.d.M., A.S. and A.M.B.d.F.; project administration, M.C.T. and A.M.B.d.F.; funding acquisition, A.S. and A.M.B.d.F. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Patient consent was waived due to this study's retrospective nature, and the fact that serum samples used in this research were sent to our laboratory by spontaneous demand for diagnostic purposes, accompanied by their respective epidemiological sheets but with patients' identification already encoded.

**Data Availability Statement:** Publicly available datasets were analyzed in this study. This data can be accessed from the NCBI BioProject: PRJNA541495.

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Supplementary Information

**Table S1**—Statistical correlations between patient's clinical outcome and immune response or days of symptoms.

| Clinical classification | Immune response | Days of Symptoms | | Clinical classification vs. Immune response (Primary/Secondary) | Clinical classification vs. Days of Symptoms |
|---|---|---|---|---|---|
| 1 | 2 | 0 | Pearson r correlation | | |
| 1 | 1 | 3 | r | -0.1602 | 0.5367 |
| 1 | 1 | 2 | 95% confidence interval | -0,384 to 0,08133 | 0,3369 to 0,6903 |
| 1 | 2 | 2 | R squared | 0.02566 | 0.2881 |
| 1 | 1 | 1 | | | |
| 1 | 1 | 3 | P value | | |
| 1 | 2 | 2 | P (two-tailed) | **0.1919** | **<0,0001** |
| 1 | 1 | 2 | P value summary | **ns** | ******** |
| 1 | 1 | 1 | Significant? (alpha = 0.05) | **No** | **Yes** |
| 1 | 1 | 1 | | | |
| 1 | 1 | 2 | Number of XY Pairs | 68 | 65 |
| 1 | 2 | 4 | | | |
| 1 | 1 | 3 | | | |
| 1 | 2 | 1 | | | |
| 1 | 1 | 4 | | | |
| 1 | 1 | 4 | | | |
| 1 | 1 | 0 | | | |
| 1 | 1 | 2 | | | |
| 1 | 2 | 2 | | | |
| 1 | 1 | 2 | | | |
| 1 | 1 | 1 | | | |
| 1 | 1 | 0 | | | |
| 1 | 2 | 5 | | | |
| 1 | 1 | 5 | | | |
| 1 | 2 | 3 | | | |
| 1 | 2 | 2 | | | |
| 1 | 2 | 2 | | | |
| 1 | 2 | 2 | | | |
| 1 | 2 | 2 | | | |
| 1 | 2 | 1 | | | |
| 1 | 2 | 3 | | | |
| 2 | 2 | 2 | | | |
| 2 | 1 | 3 | | | |
| 2 | 2 | 3 | | | |
| 2 | 1 | 2 | | | |
| 2 | 1 | 1 | | | |

| | | |
|---|---|---|
| 2 | 2 | 2 |
| 2 | 2 | 0 |
| 2 | 2 | 2 |
| 2 | 1 | 3 |
| 2 | 2 | 5 |
| 2 | 2 | 1 |
| 2 | 2 | 5 |
| 2 | 1 | 2 |
| 2 | 2 | 2 |
| 2 | 2 | 1 |
| 2 | 1 | 12 |
| 2 | 1 | 3 |
| 2 | 2 | 3 |
| 3 | 1 | 24 |
| 3 | 1 | 6 |
| 3 | 1 | 4 |
| 3 | 1 | 4 |
| 3 | 1 | 5 |
| 3 | 1 | 6 |
| 3 | 1 | 7 |
| 3 | 2 | 7 |
| 3 | 2 | 4 |
| 3 | 2 | 4 |
| 3 | 1 | 5 |
| 3 | 1 | 8 |
| 3 | 1 | 9 |
| 3 | 1 | 5 |
| 3 | 1 | 10 |
| 3 | 1 | 5 |
| 2 | 1 | ND |
| 3 | 2 | ND |
| 3 | 1 | ND |

Notes: Clinical classification: 1= Dengue fever; 2= Dengue with warning signs; 3= Severe dengue. Immune response: 1= Primary; 2= Secondary.

**Table S2**—Clinical, epidemiological, and sequencing sample characteristics.

| Sample | Collection date | Location | DofS | IR | Clinical class. | Obs. | Gender | Age | Viral Load (pfu/ul) | SRA Accession # | Consensus sequence Accession n# | Seq. platform | Total seq reads (n) | Reads length (nt) after trimming+filtering | Min Bases Quality Scores (Phred +33) after trimming+ filtering | % DENV2 specific reads | Assembly | Mapping reads after removing duplicates (%) | Genome Coverage (%) | Av. depth (x) | # iSNV/LVs after filtering |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 137 | 27/08/07 | RJ/Rio de Janeiro | 9 | P | SD | Decease | F | 50 | 8.81E+00 | SAMN11586259 | MN589858 | Nextseq 500 | 15772886 | 30-38 | >30 | 433428 | De novo | 167393 (1.06) | 99.9 | 621.05 | 130 |
| 138 | 22/11/07 | RJ/Nova Iguaçu | 2 | P | WS | - | M | 8 | 1.01E+04 | SAMN11586260 | MN589859 | Nextseq 500 | 27131548 | 30-38 | >30 | 139696 | De novo | 94978 (0.35) | 99.7 | 562.18 | 81 |
| 139 | 20/12/07 | RJ/Rio de Janeiro | 5 | P | SD | - | F | 8 | 3.42E+01 | SAMN11586261 | MN589860 | Nextseq 500 | 16123670 | 30-38 | >30 | 18224 | De novo | 9486 (0.06) | 99.8 | 318.2 | 71 |
| 140 | 17/03/08 | RJ/Rio de Janeiro | 10 | P | SD | Decease | F | 75 | 3.61E+02 | SAMN11586262 | MN589861 | Nextseq 500 | 90147034 | 30-38 | >30 | 939709 | De novo | 371634 (0.41) | 100.0 | 32.29 | 3 |
| 141 | 13/03/08 | RJ/Rio de Janeiro | 5 | S | DF | - | F | 10 | 1.16E+04 | SAMN11586263 | MN589862 | Nextseq 500 | 65074044 | 30-38 | >30 | 577370 | De novo | 185095 (0.28) | 99.9 | 1254.46 | 301 |
| 142 | 30/04/08 | RJ/Rio de Janeiro | 5 | P | DF | - | M | 10 | 1.61E+03 | SAMN11586264 | MN589863 | Nextseq 500 | 19833998 | 30-38 | >30 | 79542 | De novo | 31450 (0.16) | 99.6 | 106.22 | 11 |
| 143 | 17/03/08 | RJ/Rio de Janeiro | ND | S | SD | Decease | F | 14 | 2.81E+02 | SAMN11586265 | MN589864 | Nextseq 500 | 14534780 | 30-38 | >30 | 8021 | De novo | 5373 (0.04) | 99.5 | 18.52 | 11 |
| 144 | 07/04/08 | RJ/Rio de Janeiro | ND | P | WS | Decease | M | 85 | 7.60E+02 | SAMN11586266 | MN589865 | Nextseq 500 | 20879494 | 30-38 | >30 | 88968 | De novo | 30821 (0.15) | 99.8 | 103.97 | 8 |
| 145 | 23/12/09 | RJ/Campos dos Goytacazes | 3 | S | DF | - | F | 29 | 2.47E+02 | SAMN11586267 | MN589866 | Nextseq 500 | 21399866 | 30-38 | >30 | 560765 | De novo | 174922 (0.82) | 99.9 | 509.05 | 72 |
| 146 | 29/04/10 | RJ/Rio de Janeiro | 2 | S | DF | - | F | 50 | 2.29E+05 | SAMN11586268 | MN589867 | Nextseq 500 | 20844826 | 15-38 | >32 | 1499384 | De novo | 1014846 (4.87) | 100.0 | 3484.79 | 15 |
| 147 | 09/03/10 | RJ/Campos dos Goytacazes | 2 | S | WS | - | F | 62 | 1.73E+03 | SAMN11586269 | MN589868 | Nextseq 500 | 39112700 | 30-38 | >32 | 1499330 | De novo | 817394 (2.09) | 99.9 | 2809.06 | 7 |
| 148 | 09/03/10 | RJ/Campos dos Goytacazes | 1 | S | WS | - | F | 17 | 1.06E+02 | SAMN11586270 | MN589869 | Nextseq 500 | 50487294 | 30-38 | >30 | 848726 | De novo | 171786 (0.34) | 99.9 | 576.4 | 98 |
| 149 | 01/07/10 | RJ/Campos dos Goytacazes | 5 | P | SD | Decease | F | 5 | 4.94E+05 | SAMN11586271 | MN589870 | Nextseq 500 | 30491666 | 10-38 | >30 | 1499519 | De novo | 1134330 (3.72) | 100.0 | 3884.66 | 15 |
| 151 | 09/03/10 | RJ/Campos dos Goytacazes | 2 | S | DF | - | M | 41 | 3.82E+03 | SAMN11586272 | MN589871 | Nextseq 500 | 17411510 | 30-38 | >30 | 565107 | De novo | 188120 (1.08) | 99.9 | 634.88 | 99 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 153 | 16/02/11 | RJ/Campos dos Goytacazes | 12 | P | WS | - | F | 45 | 1.06E+02 | SAMN11586273 | MN589872 | Nextseq 500 | 13627456 | 30-38 | >30 | 227910 | De novo | 93675 (0.69) | 99.9 | 314.76 | 44 |
| 154 | 18/05/11 | RJ/Campos dos Goytacazes | 2 | S | DF | - | M | 21 | 1.56E+05 | SAMN11586274 | MN589873 | Nextseq 500 | 39170300 | 12-38 | >30 | 1499580 | De novo | 1033644 (2.64) | 100.0 | 3565.39 | 15 |
| 155 | 12/04/11 | RJ/Niteroi | 2 | S | DF | - | M | 54 | 1.66E+05 | SAMN11586275 | MN589874 | Nextseq 500 | 19430178 | 30-38 | >32 | 1449356 | De novo | 854498 (4.40) | 99.9 | 2950.21 | 10 |
| 156 | 12/04/11 | RJ/Niteroi | 3 | P | WS | - | F | 8 | 4.68E+00 | SAMN11586276 | MN589875 | Nextseq 500 | 31177520 | 30-38 | >30 | 11037 | De novo | 4557 (0.01) | 99.7 | 15.51 | 39 |
| 157 | 20/04/11 | RJ/Niteroi | 3 | S | WS | - | M | 28 | 1.59E+02 | SAMN11586277 | MN589876 | Nextseq 500 | 23385758 | 30-38 | >30 | 5399 | De novo | 2555 (0.01) | 99.4 | 8.75 | 4 |
| 158 | 04/05/11 | RJ/Campos dos Goytacazes | 1 | S | DF | - | M | 63 | 3.38E+04 | SAMN11586278 | MN589877 | Nextseq 500 | 31661364 | 30-38 | >32 | 780541 | De novo | 378903 (1.20) | 99.9 | 1309.22 | 15 |
| 159 | 04/05/11 | RJ/Campos dos Goytacazes | 3 | S | DF | - | M | 36 | 7.04E+03 | SAMN11586279 | MN589878 | Nextseq 500 | 14666016 | 30-38 | >30 | 362219 | De novo | 143364 (0.98) | 99.9 | 482.29 | 60 |
| 160 | 28/04/19 | MG/Belo Horizonte | 0 | S | DF | Pregnant | F | 24 | 6.71E+04 | SAMN17302227 | MW577809 | HiSeq4000 | 7045836 | 20-150 | >27 | 54464 | De novo | 31056 (0.44) | 98.78 | 227.23 | 212 |
| 161 | 08/05/19 | MG/Belo Horizonte | 3 | P | DF | - | F | 28 | 1.25E+04 | SAMN17302228 | MW577810 | HiSeq4000 | 9372204 | 20-150 | >28 | 25474 | De novo | 15076 (0.16) | 99.32 | 102.19 | 211 |
| 162 | 02/05/19 | MG/Luz | 2 | P | DF | - | M | 44 | 4.31E+04 | SAMN17302229 | MW577811 | HiSeq4000 | 5563880 | 20-150 | >29 | 64090 | De novo | 36958 (0.66) | 99.96 | 248.53 | 154 |
| 163 | 02/05/19 | MG/Belo Horizonte | 2 | S | DF | Pregnant | F | 28 | 8.64E+04 | SAMN17302230 | MW577812 | HiSeq4000 | 7324996 | 20-150 | >30 | 95798 | De novo | 54568 (0.74) | 99.84 | 372.25 | 113 |
| 166 | 04/04/19 | MG/Sete Lagoas | 1 | P | DF | - | M | 65 | 5.28E+05 | SAMN17302231 | MW577813 | HiSeq4000 | 8530895 | 20-150 | >31 | 7136039 | De novo | 889409 (10.4) | 100.00 | 5905.15 | 153 |
| 167 | 04/04/19 | MG/Tres Pontas | 3 | P | DF | - | F | 51 | 4.79E+05 | SAMN17302232 | MW577814 | HiSeq4000 | 5194752 | 20-150 | >30 | 101784 | De novo | 57159 (1.10) | 99.82 | 325.3 | 246 |
| 168 | 26/04/19 | MG/Bom Despacho | 2 | S | DF | - | M | 53 | 2.78E+05 | SAMN17302233 | MW577815 | HiSeq4000 | 7692344 | 20-150 | >31 | 1786647 | De novo | 394621 (5.13) | 100 | 2416.51 | 230 |
| 169 | 26/04/19 | MG/Belo Horizonte | 2 | P | DF | Pregnant | F | 30 | 2.31E+05 | SAMN17302234 | MW577816 | HiSeq4000 | 7068068 | 20-150 | >31 | 543673 | De novo | 2532813 (35.8) | 100 | 3916.72 | 88 |
| 170 | 26/04/19 | MG/Belo Horizonte | 1 | P | DF | Pregnant | F | 26 | 5.17E+04 | SAMN17302235 | MW577817 | HiSeq4000 | 6658910 | 20-150 | >31 | 1067021 | De novo | 331851 (4.98) | 100 | 2233.31 | 82 |
| 171 | 08/05/19 | MG/Sete Lagoas | 1 | P | DF | - | F | 49 | 1.35E+06 | SAMN17302236 | MW577818 | HiSeq4000 | 5399200 | 20-150 | >31 | 1261964 | De novo | 334584 (6.20) | 100 | 2117.73 | 69 |
| 172 | 09/04/19 | MG/Araxa | 2 | P | DF | - | F | 65 | 8.38E+06 | SAMN17302237 | MW577819 | HiSeq4000 | 12588952 | 20-150 | >31 | 8820723 | De novo | 894187 (7.10) | 100.00 | 4997.89 | 86 |
| 173 | 29/01/19 | RJ/Vassouras | 4 | S | DF | - | M | 42 | 2.82E+04 | SAMN17302238 | MW577820 | HiSeq4000 | 5296562 | 20-150 | >31 | 638378 | De novo | 251823 (4.75) | 99.97 | 2241.65 | 70 |
| 174 | 22/02/19 | RJ/Volta Redonda | 3 | P | DF | - | M | 59 | 2.69E+04 | SAMN17302239 | MW577821 | HiSeq4000 | 5859648 | 20-150 | >31 | 600331 | De novo | 246176 (4.20) | 100 | 2052.81 | 82 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 175 | 11/03/19 | RJ/Volta Redonda | 1 | S | DF | - | M | 37 | 9.48E+02 | SAMN17302240 | MW577822 | HiSeq4000 | 5120508 | 20-150 | >31 | 1247003 | De novo | 393332 (7.68) | 100 | 3219.11 | 70 |
| 177 | 20/05/19 | RJ/Volta Redonda | 4 | P | DF | - | M | 40 | 3.18E+04 | SAMN17302241 | MW577823 | HiSeq4000 | 5922164 | 20-150 | >31 | 1107735 | De novo | 371051 (6.27) | 100 | 3159.28 | 82 |
| 178 | 20/05/19 | RJ/Volta Redonda | 4 | P | DF | - | M | 36 | 4.98E+04 | SAMN17302242 | MW577824 | HiSeq4000 | 4965494 | 20-150 | >31 | 865866 | De novo | 324172 (6.53) | 100 | 2651.99 | 57 |
| 179 | 22/05/19 | RJ/Vassouras | 0 | P | DF | - | F | 44 | 3.40E+04 | SAMN17302243 | MW577825 | HiSeq4000 | 9098732 | 20-150 | >31 | 502267 | De novo | 230491 (2.53) | 99.99 | 1873.16 | 109 |
| 180 | 03/06/19 | RJ/Parati | 2 | P | DF | - | F | 28 | 3.08E+04 | SAMN17302244 | MW577826 | HiSeq4000 | 6555322 | 20-150 | >31 | 693676 | De novo | 304794 (4.65) | 99.98 | 2549.88 | 63 |
| 181 | 25/04/19 | MG/Belo Horizonte | 2 | S | DF | Pregnant | F | 21 | 7.84E+04 | SAMN17302245 | MW577827 | HiSeq4000 | 5372464 | 20-150 | >30 | 117045 | De novo | 70434 (1.31) | 99.84 | 467.43 | 455 |
| 182 | 03/05/19 | MG/Araxa | 2 | P | DF | Pregnant | F | 19 | 4.23E+04 | SAMN17302246 | MW577828 | HiSeq4000 | 7331342 | 20-150 | >29 | 59864 | De novo | 32693 (0.45) | 99.96 | 227.48 | 231 |
| 183 | 02/04/19 | MG/Betim | 1 | P | DF | - | M | 59 | 2.33E+05 | SAMN17302247 | MW577829 | HiSeq4000 | 9717188 | 20-150 | >30 | 119199 | De novo | 71782 (0.74) | 99.97 | 463.52 | 166 |
| 184 | 15/04/19 | MG/Arceburgo | 0 | P | DF | - | F | 54 | 3.33E+04 | SAMN17302248 | MW577830 | HiSeq4000 | 4151220 | 20-150 | >30 | 50281 | De novo | 32334 (0.78) | 99.98 | 221.22 | 150 |
| 185 | 09/03/10 | RJ/Campos dos Goytacazes | 2 | S | WS | - | F | 31 | 6.47E+05 | SAMN17302249 | MW577831 | HiSeq4000 | 6142172 | 20-150 | >31 | 2364447 | De novo | 581677 (9.47) | 100 | 4306.15 | 59 |
| 186 | 09/03/10 | RJ/Campos dos Goytacazes | 3 | P | WS | - | M | 13 | 1.62E+04 | SAMN17302250 | MW577832 | HiSeq4000 | 6221079 | 20-150 | >31 | 496840 | De novo | 218259 (3.51) | 100 | 1691.78 | 119 |
| 187 | 26/01/11 | RJ/Campos dos Goytacazes | 3 | S | WS | - | F | 24 | 6.19E+04 | SAMN17302251 | MW577833 | HiSeq4000 | 3676404 | 20-150 | >31 | 774893 | De novo | 311596 (8.48) | 100 | 2701.78 | 42 |
| 188 | 16/02/11 | RJ/Campos dos Goytacazes | 2 | P | WS | - | M | 31 | 1.32E+06 | SAMN17302252 | MW577834 | HiSeq4000 | 10747318 | 20-150 | >31 | 6225997 | De novo | 965834 (8.99) | 100 | 6030.05 | 64 |
| 189 | 30/01/10 | RJ/Campos dos Goytacazes | 1 | P | WS | - | M | 74 | 1.21E+05 | SAMN17302253 | MW577835 | HiSeq4000 | 5808192 | 20-150 | >31 | 510898 | De novo | 221952 (3.82) | 99.96 | 1827.79 | 89 |
| 190 | 19/03/19 | MG/Araxa | 2 | S | WS | - | M | 57 | 9.89E+04 | SAMN17302254 | MW577836 | HiSeq4000 | 7807864 | 20-150 | >30 | 119743 | De novo | 72519 (0.93) | 99.51 | 535.49 | 106 |
| 191 | 16/09/09 | RJ/Rio de Janeiro | 0 | S | WS | - | M | 54 | 3.78E+03 | SAMN17302255 | MW577837 | HiSeq4000 | 12312512 | 20-150 | >28 | 27354 | De novo | 14411 (0.12) | 99.58 | 97.12 | 255 |
| 192 | 16/02/11 | RJ/Campos dos Goytacazes | 2 | S | WS | - | M | 36 | 7.33E+03 | SAMN17302256 | MW577838 | HiSeq4000 | 5069358 | 20-150 | >29 | 54559 | De novo | 32996 (0.65) | 99.96 | 220.3 | 350 |
| 193 | 08/05/19 | MG/Belo Horizonte | 3 | P | WS | - | F | 24 | 2.61E+04 | SAMN17302257 | MW577839 | HiSeq4000 | 5912412 | 20-150 | >28 | 62010 | De novo | 27746 (0.47) | 99.92 | 177.48 | 256 |
| 194 | 07/04/11 | RJ/São Gonçalo | 4 | P | SD | - | M | 0.6 | 3.16E+03 | SAMN17302258 | MW577840 | HiSeq4000 | 6737846 | 20-150 | >27 | 42467 | De novo | 17852 (0.26) | 99.61 | 109.75 | 243 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 195 | 18/03/10 | SP/Santos | 4 | P | SD | - | M | 86 | 7.17E+01 | SAMN17302259 | MW577841 | HiSeq4000 | 8399360 | 20-150 | >27 | 17449 | De novo | 8273 (0.10) | 99.38 | 54.51 | 547 |
| 196 | 24/03/10 | SP/Santos | 5 | P | SD | Decease | F | 64 | 3.89E+01 | SAMN17302260 | MW577842 | HiSeq4000 | 6357071 | 20-150 | >27 | 38483 | De novo | 12355 (0.19) | 99.73 | 87.69 | 264 |
| 197 | 23/02/08 | RJ/Rio de Janeiro | 6 | P | SD | - | F | 10 | 4.17E+00 | SAMN17302261 | MW577843 | HiSeq4000 | 7517440 | 20-150 | >28 | 13173 | De novo | 8151 (0.11) | 99.33 | 52.98 | 546 |
| 198 | 23/02/08 | RJ/Rio de Janeiro | 7 | P | SD | - | F | ND | 3.79E+01 | SAMN17302262 | MW577844 | HiSeq4000 | 8822894 | 20-150 | >29 | 13741 | De novo | 8856 (0.10) | 99.65 | 56.54 | 194 |
| 199 | 05/12/08 | RJ/Duque de Caxias | 7 | S | SD | Decease | M | 7 | 2.25E+02 | SAMN17302263 | MW577845 | HiSeq4000 | 5054790 | 20-150 | >27 | 18048 | De novo | 7424 (0.15) | 99.61 | 49.9 | 204 |
| 200 | 23/04/08 | RJ/Rio de Janeiro | 4 | S | SD | Decease | F | 44 | 6.69E+02 | SAMN17302264 | MW577846 | HiSeq4000 | 4795720 | 20-150 | >28 | 23535 | De novo | 14758 (0.31) | 99.83 | 93.93 | 622 |
| 201 | 08/02/08 | RJ/Rio de Janeiro | 4 | S | SD | Decease | F | 9 | 3.23E+00 | SAMN17302265 | MW577847 | HiSeq4000 | 6061618 | 20-150 | >28 | 19052 | De novo | 10291 (0.17) | 99.06 | 64.09 | 71 |
| 202 | 04/04/10 | SP/Santos | ND | P | SD | Decease | M | 2 | 3.45E+04 | SAMN17302266 | MW577848 | HiSeq4000 | 6219700 | 20-150 | >31 | 586590 | De novo | 255529 (4.11) | 99.97 | 1928.27 | 216 |
| 203 | 16/03/08 | RJ/Rio de Janeiro | 5 | P | SD | Decease | M | 88 | 4.76E+03 | SAMN17302267 | MW577849 | HiSeq4000 | 6857444 | 20-150 | >30 | 62145 | De novo | 37657 (0.55) | 100 | 306.86 | 184 |
| 204 | 12/03/10 | RJ/Rio de Janeiro | 8 | P | SD | Decease | F | 50 | 6.75E+03 | SAMN17302268 | MW577850 | HiSeq4000 | 5760686 | 20-150 | >27 | 29708 | De novo | 12149 (0.21) | 99.92 | 84.72 | 272 |
| 205 | 18/01/10 | RJ/Campos dos Goytacazes | 1 | S | WS | - | M | 19 | 3.85E+01 | SAMN17302269 | MW577851 | HiSeq4000 | 3727624 | 20-150 | >29 | 15116 | De novo | 10508 (0.28) | 98.69 | 71.39 | 599 |
| 206 | 02/03/10 | SP/Santos | 5 | S | WS | - | M | 21 | 5.03E+00 | SAMN17302270 | MW577852 | HiSeq4000 | 8501734 | 20-150 | >28 | 21459 | De novo | 10117 (0.12) | 99.60 | 65.11 | 135 |
| 207 | 17/03/09 | RJ/Niteroi | 5 | S | WS | - | M | 60 | 1.08E+01 | SAMN17302271 | MW577853 | HiSeq4000 | 7898602 | 20-150 | >28 | 9693 | De novo | 5803 (0.07) | 98.62 | 38.07 | 190 |
| 208 | 07/02/08 | RJ/Rio de Janeiro | 24 | P | SD | Decease | M | 15 | 2.86E+00 | SAMN17302272 | MW577854 | HiSeq4000 | 6773006 | 20-150 | >27 | 14490 | De novo | 8089 (0.12) | 99.23 | 51.07 | 104 |
| 209 | 22/03/10 | SP/Santos | 6 | P | SD | Decease | F | 12 | 7.52E+00 | SAMN17302273 | MW577855 | HiSeq4000 | 6010820 | 20-150 | >27 | 13525 | De novo | 6787 (0.11) | 99.01 | 44.75 | 161 |

Clinical class: clinical classification; DF: dengue fever; WS: dengue with warning signs; SD: severe dengue; Obs: observations; M: male; F: female; RJ: state of Rio de Janeiro; SP: state of São Paulo; MG: state of Minas Gerais; VL: viral load; pfu: plaque formation unit; IR: immune classification; P: primary infection; S: secondary infection; ND: information not determined; Seq: sequencing; nt: nucleotide; Av: average; iSNV: intrahost sigle nucleotide variant; iLV: intrahost long variant.

**Table S3**—Single nucleotide variants (iSNVs) + Long variants (iLVs, accounting for insertions+deletions) constituting viral mutant swarm of each sample. POS: position; REF: reference; ALT: altered; Aa: aminoacid; fs: frameshift.

| **Sample** | **137** | | | | | |
|---|---|---|---|---|---|---|
| Clinical classif | SD | | | | | |
| Imune resp. | Primary | | | | | |
| Viral load (pfu/ml) | 8.81E+00 | | | | | |
| DofS | 9 | | | | | |
| | Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| | 5UTR | 67 | A | T | - | 1.99 |
| | 5UTR | 70 | G | GT | - | 1.55 |
| | C | 210 | G | C | - | 1.22 |
| | C | 226 | T | A | Leu44Ile | 1.19 |
| | C | 229 | A | T | Lys45* | 0.97 |
| | C | 363 | A | T | - | 1.35 |
| | C | 367 | A | C | Met91Leu | 0.75 |
| | C | 369 | G | C | Met91Ile | 1.00 |
| | C | 372 | G | C | - | 0.98 |
| | C | 399 | A | T | - | 0.90 |
| | prM/M | 472 | A | T | Met12Leu | 1.33 |
| | prM/M | 522 | G | C | Glu28Asp | 1.31 |
| | prM/M | 523 | G | C | Asp29His | 1.03 |
| | prM/M | 529 | G | C | Val31Leu | 1.48 |
| | prM/M | 548 | T | A | Met37Lys | 1.08 |
| | prM/M | 582 | A | T | - | 1.34 |
| | prM/M | 731 | A | T | His98Leu | 1.10 |
| | prM/M | 741 | G | C | Met101Ile | 1.03 |
| | prM/M | 888 | A | T | Gln150His | 1.69 |
| | E | 950 | G | C | Gly5Ala | 1.00 |
| | E | 1127 | A | T | Lys64Met | 1.14 |
| | E | 1289 | T | A | Met118Lys | 1.25 |
| | E | 1297 | T | A | Cys121Ser | 0.96 |
| | E | 1467 | C | G | - | 1.26 |
| | E | 1545 | T | C | - | 11.29 |
| | E | 1599 | G | A | - | 23.40 |
| | E | 1641 | G | C | Glu235Asp | 3.43 |
| | E | 1724 | C | A | Ala263Glu | 1.69 |
| | E | 1885 | C | G | His317Asp | 1.35 |
| | E | 1903 | A | T | Arg323* | 1.55 |
| | E | 2065 | T | A | Tyr377Asn | 1.22 |
| | E | 2226 | A | T | - | 1.12 |
| | E | 2275 | G | C | Ala447Pro | 1.40 |
| | NS1 | 2492 | A | T | Asn24Ile | 1.70 |
| | NS1 | 2505 | G | C | Trp28Cys | 2.09 |
| | NS1 | 2667 | T | G | Asn82Lys | 1.38 |
| | NS1 | 2687 | T | A | Met89Lys | 1.77 |
| | NS1 | 2750 | A | T | Glu110Val | 1.30 |
| | NS1 | 3026 | T | A | Ile202Lys | 1.23 |
| | NS1 | 3114 | C | G | - | 1.31 |
| | NS1 | 3122 | A | T | Asn234Ile | 1.58 |
| | NS1 | 3133 | G | C | Glu238Gln | 1.62 |
| | NS1 | 3152 | C | CA | Asn245fs | 1.72 |
| | NS1 | 3335 | G | C | Gly305Ala | 1.98 |
| | NS1 | 3394 | G | C | Gly325Arg | 1.83 |
| | NS2A | 3689 | G | C | Gly71Ala | 1.44 |
| | NS2A | 3785 | T | G | Met103Arg | 0.87 |
| | NS3 | 5861 | T | A | Met447Lys | 3.79 |
| | NS3 | 5873 | A | G | His451Arg | 3.98 |
| | NS3 | 5915 | C | CA | Asn467fs | 6.95 |
| | NS3 | 6018 | C | G | Asn499Lys | 1.87 |
| | NS3 | 6049 | G | C | Glu510Gln | 1.78 |
| | NS4A | 6546 | A | G | - | 1.23 |
| | NS4A | 6573 | A | T | - | 1.69 |
| | NS4A | 6652 | A | T | Ile93Phe | 1.27 |
| | NS4A | 6711 | G | GT | Leu115fs | 1.16 |
| | NS4B | 6901 | A | T | Asn26Tyr | 1.87 |
| | NS4B | 6930 | A | T | - | 1.37 |
| | NS4B | 6996 | A | T | Glu57Asp | 1.44 |
| | NS4B | 7011 | T | C | - | 11.18 |
| | NS4B | 7120 | T | A | Tyr99Asn | 1.12 |
| | NS4B | 7131 | C | A | - | 1.30 |
| | NS4B | 7277 | C | G | Thr151Arg | 1.80 |
| | NS4B | 7282 | A | T | Ile153Phe | 1.27 |
| | NS5 | 7752 | A | G | - | 2.00 |
| | NS5 | 7791 | A | G | - | 3.91 |
| | NS5 | 7994 | C | G | Thr142Arg | 1.75 |
| | NS5 | 8018 | C | G | Ser150* | 1.58 |
| | NS5 | 8104 | T | G | Cys179Gly | 2.00 |
| | NS5 | 8143 | G | GA | Met194fs | 0.55 |
| | NS5 | 8204 | G | T | Arg212Leu | 1.47 |
| | NS5 | 8238 | T | A | Asn223Lys | 1.65 |
| | NS5 | 8272 | A | T | Ile235Phe | 0.86 |
| | NS5 | 8342 | T | A | Leu258His | 1.33 |
| | NS5 | 8383 | C | G | Pro272Ala | 1.23 |
| | NS5 | 8514 | A | T | - | 1.41 |
| | NS5 | 8518 | G | C | Ala317Pro | 1.21 |
| | NS5 | 8613 | A | G | - | 1.35 |
| | NS5 | 8650 | A | T | Thr361Ser | 1.22 |
| | NS5 | 8659 | C | G | - | 1.34 |
| | NS5 | 8787 | T | A | Asn406Lys | 1.42 |
| | NS5 | 8906 | A | T | Lys446Met | 0.85 |
| | NS5 | 8908 | T | A | Cys447Ser | 1.18 |
| | NS5 | 8910 | T | A | Cys447* | 1.25 |
| | NS5 | 8913 | A | T | Glu448Asp | 1.80 |
| | NS5 | 8932 | A | T | Met455Leu | 0.74 |
| | NS5 | 8998 | A | T | Met477Leu | 1.09 |
| | NS5 | 9004 | C | G | Leu479Val | 1.76 |
| | NS5 | 9074 | A | G | Asn502Ser | 2.28 |
| | NS5 | 9093 | A | G | - | 2.13 |
| | NS5 | 9099 | A | G | - | 4.44 |
| | NS5 | 9114 | A | G | - | 2.99 |
| | NS5 | 9239 | T | A | Met557Lys | 1.94 |
| | NS5 | 9463 | A | C | Ile632Leu | 1.40 |
| | NS5 | 9503 | A | T | Asn645Ile | 1.51 |
| | NS5 | 9569 | T | A | Val667Glu | 1.15 |
| | NS5 | 9593 | C | G | Ala675Gly | 1.38 |
| | NS5 | 9595 | A | T | Ser676Cys | 1.29 |
| | NS5 | 9705 | T | TC | Phe713fs | 0.62 |
| | NS5 | 9744 | A | G | - | 2.19 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2A | 3918 | G | C | - | 1.28 | NS5 | 9899 | A | G | Asn777Ser | 6.02 |
| NS2B | 4259 | T | A | Val43Glu | 2.39 | NS5 | 9912 | G | A | - | 6.25 |
| NS2B | 4260 | G | C | - | 2.39 | NS5 | 10089 | A | G | - | 12.48 |
| NS2B | 4462 | C | G | Pro111Ala | 1.74 | NS5 | 10142 | C | G | Thr858Ser | 1.28 |
| NS3 | 4566 | A | G | - | 2.51 | NS5 | 10167 | A | T | - | 0.92 |
| NS3 | 4604 | G | C | Arg28Thr | 2.17 | NS5 | 10196 | A | T | Asn876Ile | 1.19 |
| NS3 | 4761 | A | T | - | 1.15 | NS5 | 10217 | T | A | Met883Lys | 1.23 |
| NS3 | 4848 | C | G | - | 1.67 | NS5 | 10218 | G | C | Met883Ile | 1.07 |
| NS3 | 5086 | A | T | Thr189Ser | 1.56 | 3UTR | 10286 | C | G | | 1.35 |
| NS3 | 5089 | A | T | Ile190Phe | 1.75 | 3UTR | 10314 | A | T | | 1.31 |
| NS3 | 5092 | A | T | Met191Leu | 2.12 | 3UTR | 10566 | T | C | | 10.17 |
| NS3 | 5376 | A | G | - | 1.52 | 3UTR | 10611 | C | CA | | 12.50 |
| NS3 | 5493 | A | T | - | 1.64 | 3UTR | 10611 | C | CAA | | 41.25 |
| NS3 | 5503 | A | T | Ser328Cys | 1.46 | 3UTR | 10611 | C | CAAA | | 10.00 |
| NS3 | 5754 | A | T | - | 2.73 | 3UTR | 10611 | C | CAAAA | | 3.75 |

| **138** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WS | | | | | | NS2B | 4216 | A | T | Thr29Ser | 1.99 |
| Primary | | | | | | NS3 | 4643 | A | T | Tyr41Phe | 1.64 |
| 1.01E+04 | | | | | | NS3 | 4829 | G | GA | Asn105fs | 1.84 |
| 2 | | | | | | NS3 | 5024 | C | G | Thr168Ser | 0.94 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5154 | C | G | - | 1.37 |
| 5UTR | 70 | G | GT | - | 2.72 | NS3 | 5176 | T | A | Leu219Ile | 1.44 |
| C | 240 | G | C | Met48Ile | 1.51 | NS3 | 5447 | C | G | Ala309Gly | 1.43 |
| C | 246 | T | A | - | 1.33 | NS3 | 5522 | A | T | Asp334Val | 1.49 |
| C | 347 | T | A | Phe84Tyr | 1.46 | NS3 | 5567 | A | T | Glu349Val | 1.59 |
| prM/M | 689 | G | C | Gly84Ala | 2.40 | NS3 | 5659 | A | T | Lys380* | 1.96 |
| prM/M | 703 | G | GA | Arg91fs | 2.16 | NS3 | 5694 | C | G | Asp391Glu | 2.06 |
| E | 1040 | C | CA | Asn37fs | 4.17 | NS3 | 5696 | C | A | Ser392Tyr | 1.14 |
| E | 1102 | C | G | Leu56Val | 1.39 | NS3 | 5699 | A | T | Glu393Val | 1.04 |
| E | 1357 | A | T | Ile141Leu | 1.28 | NS3 | 5718 | C | G | - | 2.23 |
| E | 1833 | C | G | Tyr299* | 1.54 | NS3 | 5832 | C | G | - | 2.35 |
| E | 1905 | A | T | Arg323Ser | 1.14 | NS3 | 5870 | C | G | Thr450Ser | 2.70 |
| E | 1984 | C | A | Arg350Ser | 1.69 | NS3 | 5915 | C | CA | Asn467fs | 10.91 |
| E | 1990 | A | T | Ile352Phe | 1.87 | NS4A | 6645 | A | T | - | 1.55 |
| NS1 | 2424 | T | A | Asp1Glu | 1.35 | NS4A | 6658 | C | G | Leu95Val | 1.53 |
| NS1 | 2511 | G | C | Glu30Asp | 1.48 | NS4A-2K | 6789 | C | G | - | 1.95 |
| NS1 | 2533 | T | A | Ser38Thr | 0.87 | NS4B | 6949 | G | C | Ala42Pro | 1.78 |
| NS1 | 2605 | A | T | Arg62* | 1.43 | NS4B | 6955 | G | T | Ala44Ser | 1.43 |
| NS1 | 2611 | G | C | Glu64Gln | 1.38 | NS4B | 6983 | G | T | Arg53Ile | 2.81 |
| NS1 | 2643 | A | T | Glu74Asp | 1.58 | NS4B | 7180 | G | C | Ala119Pro | 1.68 |
| NS1 | 2650 | C | G | His77Asp | 1.28 | NS4B | 7273 | A | T | Ile150Leu | 2.01 |
| NS1 | 2665 | A | T | Asn82Tyr | 1.18 | NS4B | 7333 | C | G | Gln170Glu | 2.24 |
| NS1 | 2680 | A | T | Thr87Ser | 1.04 | NS4B | 7363 | C | G | Gln180Glu | 1.65 |
| NS1 | 2683 | A | T | Ile88Phe | 0.98 | NS4B | 7385 | C | G | Thr187Arg | 2.76 |
| NS1 | 2797 | A | T | Thr126Ser | 1.66 | NS5 | 8306 | A | T | Lys246Ile | 1.35 |
| NS1 | 2962 | C | T | - | 30.04 | NS5 | 8442 | A | T | - | 2.20 |
| NS1 | 3152 | C | CA | Asn245fs | 4.36 | NS5 | 8847 | T | A | - | 2.17 |
| NS1 | 3237 | G | C | Lys272Asn | 1.82 | NS5 | 8926 | A | AC | Asn453fs | 0.77 |
| NS1 | 3307 | C | G | Pro296Ala | 1.76 | NS5 | 9112 | C | G | Leu515Val | 2.45 |
| NS1 | 3418 | A | T | Met333Leu | 1.69 | NS5 | 9550 | A | T | Ser661Cys | 1.96 |
| NS1 | 3446 | A | T | Glu342Val | 2.16 | NS5 | 9705 | T | TC | Phe713fs | 0.53 |
| NS2A | 3849 | T | A | - | 1.13 | NS5 | 9736 | G | C | Val723Leu | 1.22 |
| NS2A | 3985 | A | T | Thr170Ser | 1.68 | NS5 | 9757 | A | T | Asn730Tyr | 1.23 |
| NS2A | 3998 | C | A | Ala174Glu | 1.53 | NS5 | 9847 | C | G | Gln760Glu | 1.09 |
| NS2A | 4013 | C | G | Pro179Arg | 1.68 | NS5 | 9926 | A | T | His786Leu | 1.34 |
| NS2A | 4019 | T | A | Leu181His | 1.53 | NS5 | 10015 | G | C | Val816Leu | 1.53 |
| NS2A | 4023 | A | T | Leu182Phe | 1.12 | NS5 | 10066 | T | A | Trp833Arg | 1.75 |
| NS2A | 4045 | G | C | Asp190His | 1.13 | NS5 | 10167 | A | T | - | 2.11 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2B | 4205 | A | T | Asp25Val | 1.06 | NS5 | 10182 | A | T | Arg871Ser | 1.96 |

| **139** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SD | | | | | | NS3 | 4691 | T | A | Val57Asp | 4.01 |
| Primary | | | | | | NS3 | 4908 | C | T | - | 3.50 |
| 3.42E+01 | | | | | | NS3 | 4986 | C | G | - | 1.38 |
| 5 | | | | | | NS3 | 5092 | A | T | Met191Leu | 3.57 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5186 | C | G | Ala222Gly | 5.26 |
| 5UTR | 67 | A | T | - | 2.67 | NS3 | 5192 | C | G | Thr224Ser | 6.90 |
| C | 110 | G | GA | Lys5fs | 3.21 | NS3 | 5377 | G | C | Ala286Pro | 3.15 |
| C | 235 | T | A | Phe47Ile | 1.43 | NS3 | 5531 | G | T | Arg337Ile | 4.78 |
| C | 399 | A | T | - | 1.04 | NS3 | 5572 | G | C | Val351Leu | 2.61 |
| C | 424 | C | G | Pro110Ala | 1.96 | NS3 | 5699 | A | T | Glu393Val | 2.51 |
| prM/M | 506 | T | C | Leu23Pro | 1.17 | NS3 | 6041 | G | C | Ser507Thr | 2.09 |
| prM/M | 539 | G | C | Cys34Ser | 1.71 | NS3 | 6308 | T | A | Leu596* | 2.58 |
| prM/M | 677 | G | C | Cys80Ser | 2.23 | NS4A | 6611 | G | C | Gly79Ala | 2.75 |
| prM/M | 683 | C | G | Thr82Ser | 2.61 | NS4A | 6617 | T | A | Met81Lys | 2.86 |
| prM/M | 703 | G | GA | Arg91fs | 1.24 | NS4A | 6711 | G | GT | Leu115fs | 2.08 |
| prM/M | 770 | T | A | Met111Lys | 2.50 | NS4B | 6907 | C | G | Leu28Val | 3.28 |
| prM/M | 801 | G | T | Gln121His | 2.06 | NS4B | 7049 | T | C | Leu75Ser | 1.46 |
| prM/M | 878 | C | G | Thr147Arg | 2.31 | NS4B | 7537 | T | A | Ser238Thr | 2.35 |
| E | 1018 | G | C | Gly28Arg | 2.02 | NS5 | 8238 | T | A | Asn223Lys | 1.73 |
| E | 1112 | A | T | Tyr59Phe | 1.63 | NS5 | 8398 | A | T | Ile277Phe | 2.33 |
| E | 1265 | A | T | Lys110 Met | 1.88 | NS5 | 8623 | C | G | Gln352Glu | 1.55 |
| E | 1696 | G | C | Gly254Arg | 4.92 | NS5 | 8744 | G | C | Arg392Thr | 1.30 |
| E | 1853 | T | A | Phe306Tyr | 1.82 | NS5 | 8913 | A | T | Glu448Asp | 1.97 |
| E | 1907 | T | A | Val324Glu | 2.16 | NS5 | 8932 | A | T | Met455Leu | 1.27 |
| E | 2035 | A | T | Ile367Leu | 2.11 | NS5 | 8986 | G | GCCACATGTA | Ala474insThrCysThr | 0.57 |
| E | 2155 | A | T | Arg407Trp | 1.83 | NS5 | 8991 | A | T | - | 1.51 |
| E | 2295 | G | C | Trp453Cys | 2.39 | NS5 | 9182 | G | C | Trp538Ser | 2.41 |
| NS1 | 2505 | G | C | Trp28Cys | 1.79 | NS5 | 9293 | A | T | Asn575Ile | 1.71 |
| NS1 | 2511 | G | C | Glu30Asp | 2.35 | NS5 | 9324 | A | T | - | 1.35 |
| NS1 | 2656 | C | T | - | 39.64 | NS5 | 9330 | C | G | - | 1.61 |
| NS1 | 3059 | AG | A | Ala213fs | 0.77 | NS5 | 9437 | T | A | Met623Lys | 2.14 |
| NS1 | 3152 | C | CA | Asn245fs | 1.40 | NS5 | 9569 | T | A | Val667Glu | 1.59 |
| NS1 | 3375 | A | T | Leu318Phe | 1.39 | NS5 | 9634 | A | T | Lys689* | 1.20 |
| NS2A | 3689 | G | C | Gly71Ala | 1.94 | NS5 | 9735 | C | G | - | 3.37 |
| NS2A | 3827 | C | G | Pro117Arg | 1.51 | NS5 | 9999 | G | T | - | 10.26 |
| NS2A | 3838 | C | G | Leu121Val | 1.84 | NS5 | 10205 | A | C | Tyr879Ser | 2.05 |
| NS2A | 3848 | C | G | Thr124Ser | 2.43 | NS5 | 10212 | C | G | Asp881Glu | 2.00 |
| NS3 | 4599 | G | C | Lys26Asn | 3.15 | 3UTR | 10353 | C | G | - | 3.02 |

| **140** | | | | | | NS3 | 4585 | G | T | Ala22Ser | 1.69 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SD | | | | | | NS3 | 4649 | A | T | Glu43Val | 1.22 |
| Primary | | | | | | NS3 | 4891 | G | C | Gly124Arg | 1.18 |
| 3.61E+02 | | | | | | NS3 | 4895 | C | G | Ala125Gly | 1.04 |
| 10 | | | | | | NS3 | 4896 | T | A | - | 1.05 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 4897 | G | C | Val126Leu | 1.16 |
| E | 2298 | T | A | - | 31.58 | NS3 | 5046 | T | A | Asn175Lys | 1.17 |
| NS3 | 5915 | C | CA | Asn467fs | 9.09 | NS3 | 5086 | A | T | Thr189Ser | 1.52 |
| NS5 | 9827 | G | T | Cys753Phe | 9.38 | NS3 | 5102 | A | T | His194Leu | 1.06 |
| **141** | | | | | | NS3 | 5180 | T | A | Ile220Asn | 1.22 |
| DF | | | | | | NS3 | 5186 | C | G | Ala222Gly | 0.90 |
| Secondary | | | | | | NS3 | 5188 | C | G | Pro223Ala | 1.02 |

| 1.16E+04 | | | | | |
|---|---|---|---|---|---|
| 5 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 214 | G | C | Gly40Arg | 1.41 |
| C | 246 | T | A | - | 2.16 |
| C | 357 | G | C | Glu87Asp | 1.58 |
| C | 392 | G | C | Arg99Pro | 0.92 |
| C | 428 | C | G | Thr111Arg | 1.22 |
| prM/M | 462 | A | T | - | 0.92 |
| prM/M | 481 | G | C | Gly15Arg | 1.56 |
| prM/M | 517 | A | T | Thr27Ser | 1.50 |
| prM/M | 523 | G | C | Asp29His | 0.93 |
| prM/M | 529 | G | C | Val31Leu | 1.08 |
| prM/M | 538 | T | A | Cys34Ser | 1.08 |
| prM/M | 547 | A | T | Met37Leu | 0.69 |
| prM/M | 582 | A | T | - | 1.12 |
| prM/M | 584 | T | A | Ile49Asn | 0.82 |
| prM/M | 593 | A | T | Lys52Met | 0.80 |
| prM/M | 615 | T | A | Asn59Lys | 0.95 |
| prM/M | 683 | C | G | Thr82Ser | 1.14 |
| prM/M | 703 | G | GA | Arg91fs | 0.83 |
| prM/M | 732 | T | A | His98Gln | 1.04 |
| prM/M | 762 | A | T | Glu108Asp | 1.12 |
| prM/M | 769 | A | T | Met111Leu | 0.94 |
| prM/M | 860 | C | G | Ala141Gly | 1.11 |
| prM/M | 878 | C | G | Thr147Arg | 1.45 |
| prM/M | 884 | T | A | Phe149Tyr | 1.42 |
| prM/M | 888 | A | T | Gln150His | 0.96 |
| E | 937 | A | C | Met1Leu | 0.98 |
| E | 945 | C | G | Cys3Trp | 1.02 |
| E | 964 | G | C | Asp10His | 0.89 |
| E | 1040 | C | CA | Asn37fs | 2.16 |
| E | 1056 | A | T | - | 1.24 |
| E | 1101 | T | A | - | 0.71 |
| E | 1102 | C | G | Leu56Val | 0.90 |
| E | 1112 | A | T | Tyr59Phe | 1.34 |
| E | 1127 | A | T | Lys64Met | 1.68 |
| E | 1164 | A | T | - | 1.45 |
| E | 1174 | C | G | Pro80Ala | 1.35 |
| E | 1214 | A | T | Lys93Ile | 0.80 |
| E | 1224 | G | C | Met96Ile | 1.67 |
| E | 1279 | A | T | Thr115Ser | 1.42 |
| E | 1285 | G | C | Ala117Pro | 1.61 |
| E | 1296 | A | T | Arg120Ser | 0.89 |
| E | 1297 | T | A | Cys121Ser | 0.92 |
| E | 1367 | A | T | His144Leu | 1.42 |
| E | 1368 | C | G | His144Gln | 0.80 |
| E | 1377 | A | T | Glu147Asp | 0.81 |
| E | 1449 | A | T | - | 0.86 |
| E | 1473 | C | G | - | 0.89 |
| E | 1516 | A | T | Asn194Tyr | 1.10 |
| E | 1521 | G | C | Glu195Asp | 0.90 |
| E | 1522 | A | T | Met196Leu | 1.08 |
| E | 1560 | G | C | - | 0.88 |
| E | 1585 | C | G | Pro217Ala | 0.79 |
| E | 1596 | G | C | Trp220Cys | 0.97 |
| E | 1611 | T | A | Asp225Glu | 0.72 |
| E | 1612 | A | T | Thr226Ser | 0.70 |
| E | 1620 | A | T | - | 0.82 |
| E | 1625 | A | T | Asn230Ile | 1.03 |
| NS3 | 5211 | A | T | Glu230Asp | 1.26 |
| NS3 | 5225 | T | A | Leu235His | 0.82 |
| NS3 | 5234 | T | A | Leu238His | 0.81 |
| NS3 | 5299 | A | T | Met260Leu | 1.09 |
| NS3 | 5324 | G | C | Arg268Thr | 0.79 |
| NS3 | 5330 | T | A | Leu270Gln | 1.11 |
| NS3 | 5340 | C | T | - | 5.75 |
| NS3 | 5419 | A | T | Ile300Phe | 0.99 |
| NS3 | 5425 | A | T | Thr302Ser | 1.70 |
| NS3 | 5474 | C | G | Pro318Arg | 1.23 |
| NS3 | 5493 | A | T | - | 0.84 |
| NS3 | 5499 | T | A | - | 0.78 |
| NS3 | 5510 | C | G | Ala330Gly | 0.88 |
| NS3 | 5632 | A | T | Ile371Leu | 1.28 |
| NS3 | 5672 | A | T | Gln384Leu | 1.13 |
| NS3 | 5694 | C | G | Asp391Glu | 1.65 |
| NS3 | 5702 | A | C | Tyr394Ser | 0.92 |
| NS3 | 5706 | T | A | - | 1.10 |
| NS3 | 5710 | A | T | Thr397Ser | 1.04 |
| NS3 | 5711 | C | G | Thr397Ser | 1.11 |
| NS3 | 5884 | G | T | Ala455Ser | 1.42 |
| NS3 | 5886 | G | C | - | 1.72 |
| NS3 | 5897 | G | C | Gly459Ala | 1.42 |
| NS3 | 5915 | C | CA | Asn467fs | 7.84 |
| NS3 | 5971 | G | C | Asp484His | 1.00 |
| NS3 | 6041 | G | C | Ser507Thr | 1.09 |
| NS3 | 6047 | T | A | Phe509Tyr | 1.79 |
| NS3 | 6071 | A | T | Asp517Val | 1.03 |
| NS3 | 6192 | C | G | Tyr557* | 1.78 |
| NS3 | 6214 | G | C | Asp565His | 1.14 |
| NS3 | 6240 | G | T | - | 1.52 |
| NS3 | 6242 | A | T | Glu574Val | 1.04 |
| NS3 | 6335 | T | A | Leu605Gln | 1.06 |
| NS4A | 6475 | G | C | Ala34Pro | 0.97 |
| NS4A | 6504 | T | A | His43Gln | 1.04 |
| NS4A | 6616 | A | T | Met81Leu | 0.85 |
| NS4A | 6654 | T | C | - | 1.11 |
| NS4A | 6664 | T | A | Tyr97Asn | 6.15 |
| NS4A | 6668 | C | G | Ala98Gly | 1.04 |
| NS4A | 6674 | T | A | Ile100Lys | 0.94 |
| NS4A | 6678 | A | T | Gln101His | 1.17 |
| NS4A | 6726 | T | C | - | 8.54 |
| NS4A | 6730 | C | G | Leu119Val | 1.79 |
| NS4A | 6736 | C | G | Pro121Ala | 2.03 |
| NS4B | 6931 | T | A | Ser36Thr | 1.10 |
| NS4B | 6979 | T | A | Leu52Met | 1.09 |
| NS4B | 7006 | G | C | Val61Leu | 0.93 |
| NS4B | 7039 | G | C | Ala72Pro | 0.83 |
| NS4B | 7041 | T | A | - | 0.94 |
| NS4B | 7145 | T | A | Leu107His | 0.75 |
| NS4B | 7272 | A | T | - | 0.71 |
| NS4B | 7276 | A | T | Thr151Ser | 1.25 |
| NS4B | 7283 | T | A | Ile153Asn | 0.96 |
| NS4B | 7306 | G | C | Asp161His | 1.09 |
| NS4B | 7332 | A | T | - | 1.42 |
| NS4B | 7336 | G | C | Val171Leu | 0.99 |
| NS4B | 7339 | A | T | Met172Leu | 0.95 |
| NS4B | 7348 | A | T | Ile175Phe | 0.90 |
| NS4B | 7378 | A | T | Arg185Trp | 0.79 |
| NS4B | 7433 | C | G | Thr203Arg | 1.59 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1631 | T | A | Ile232Lys | 1.12 | NS4B | 7539 | C | G | - | 1.65 |
| E | 1635 | G | C | Gln233His | 1.30 | NS4B | 7560 | C | A | Asn245Lys | 1.02 |
| E | 1686 | C | G | - | 1.18 | NS5 | 7570 | G | T | Gly1* | 1.17 |
| E | 1702 | C | G | Gln256Glu | 0.97 | NS5 | 7582 | G | C | Val5Leu | 1.80 |
| E | 1706 | A | T | Glu257Val | 0.85 | NS5 | 7689 | A | T | Leu40Phe | 1.22 |
| E | 1724 | C | G | Ala263Gly | 1.00 | NS5 | 7730 | C | G | Ala54Gly | 1.12 |
| E | 1729 | A | T | Thr265Ser | 1.01 | NS5 | 7835 | A | T | Tyr89Phe | 1.60 |
| E | 1731 | A | C | - | 0.75 | NS5 | 7866 | A | C | Glu99Asp | 0.94 |
| E | 1740 | A | T | - | 0.91 | NS5 | 7911 | C | G | Ile114Met | 1.24 |
| E | 1789 | T | A | Cys285Ser | 0.99 | NS5 | 8004 | T | A | Cys145* | 1.12 |
| E | 1821 | A | T | Lys295Asn | 1.05 | NS5 | 8004 | T | C | - | 1.36 |
| E | 1827 | G | C | Met297Ile | 1.37 | NS5 | 8071 | G | C | Val168Leu | 1.15 |
| E | 1838 | T | A | Met301Lys | 0.91 | NS5 | 8130 | G | C | Met187Ile | 0.74 |
| E | 1853 | T | A | Phe306Tyr | 1.67 | NS5 | 8162 | A | T | Gln198Leu | 0.78 |
| E | 1885 | C | G | His317Asp | 1.21 | NS5 | 8217 | T | A | His216Gln | 0.76 |
| E | 1886 | A | T | His317Leu | 0.89 | NS5 | 8230 | G | C | Val221Leu | 0.99 |
| E | 1906 | G | C | Val324Leu | 0.72 | NS5 | 8238 | T | A | Asn223Lys | 0.92 |
| E | 1931 | C | G | Pro332Arg | 0.97 | NS5 | 8243 | C | G | Ser225Cys | 0.96 |
| E | 1944 | T | A | - | 1.12 | NS5 | 8263 | G | C | Val232Leu | 0.87 |
| E | 1988 | T | A | Leu351Gln | 1.00 | NS5 | 8278 | A | T | Arg237Trp | 0.86 |
| E | 1990 | A | T | Ile352Phe | 1.19 | NS5 | 8312 | A | T | Lys248Met | 1.34 |
| E | 2088 | G | T | - | 1.55 | NS5 | 8322 | C | G | - | 1.45 |
| E | 2155 | A | T | Arg407Trp | 1.20 | NS5 | 8334 | T | A | Asp255Glu | 0.99 |
| E | 2161 | G | C | Ala409Pro | 1.23 | NS5 | 8383 | C | G | Pro272Ala | 0.98 |
| E | 2214 | A | T | - | 1.14 | NS5 | 8393 | A | T | Asp275Val | 1.21 |
| E | 2275 | G | C | Ala447Pro | 0.88 | NS5 | 8442 | A | T | - | 1.16 |
| E | 2342 | A | T | Asn469Ile | 0.79 | NS5 | 8472 | A | T | Lys301Asn | 1.28 |
| E | 2399 | A | T | Tyr488Phe | 0.91 | NS5 | 8524 | T | A | Ser319Thr | 1.43 |
| NS1 | 2480 | T | A | Phe20Tyr | 0.81 | NS5 | 8534 | A | T | Asn322Ile | 1.10 |
| NS1 | 2493 | C | G | Asn24Lys | 0.77 | NS5 | 8536 | G | C | Gly323Arg | 1.17 |
| NS1 | 2494 | G | C | Val25Leu | 0.97 | NS5 | 8684 | A | T | Lys372Ile | 2.39 |
| NS1 | 2495 | T | A | Val25Glu | 1.07 | NS5 | 8721 | A | T | Glu384Asp | 1.15 |
| NS1 | 2500 | A | T | Thr27Ser | 0.95 | NS5 | 8736 | G | C | Lys389Asn | 1.21 |
| NS1 | 2506 | A | T | Thr29Ser | 0.89 | NS5 | 8742 | T | A | - | 1.23 |
| NS1 | 2510 | A | T | Glu30Val | 0.84 | NS5 | 8919 | T | A | Cys450* | 1.36 |
| NS1 | 2511 | G | C | Glu30Asp | 1.37 | NS5 | 8972 | C | G | Ala468Gly | 1.25 |
| NS1 | 2517 | T | A | Tyr32* | 1.54 | NS5 | 8982 | C | G | Ser471Arg | 1.44 |
| NS1 | 2569 | C | G | His50Asp | 0.81 | NS5 | 9004 | C | G | Leu479Val | 1.56 |
| NS1 | 2595 | C | G | - | 1.18 | NS5 | 9106 | C | G | His513Asp | 1.24 |
| NS1 | 2637 | A | T | - | 1.15 | NS5 | 9112 | C | G | Leu515Val | 2.10 |
| NS1 | 2643 | A | T | Glu74Asp | 1.44 | NS5 | 9142 | A | T | Lys525* | 1.32 |
| NS1 | 2658 | A | T | - | 0.68 | NS5 | 9489 | A | T | Glu640Asp | 1.38 |
| NS1 | 2664 | A | T | Glu81Asp | 1.16 | NS5 | 9502 | A | T | Asn645Tyr | 1.40 |
| NS1 | 2786 | A | T | Lys122Ile | 1.34 | NS5 | 9565 | G | C | Val666Leu | 1.14 |
| NS1 | 2971 | A | C | Met184Leu | 1.16 | NS5 | 9603 | A | T | Leu678Phe | 0.81 |
| NS1 | 3011 | A | T | Asp197Val | 1.18 | NS5 | 9628 | G | C | Ile687Leu | 0.97 |
| NS1 | 3074 | T | A | Ile218Asn | 1.11 | NS5 | 9634 | A | T | Lys689* | 0.87 |
| NS1 | 3123 | T | A | Asn234Lys | 0.95 | NS5 | 9649 | T | A | Trp694Arg | 1.18 |
| NS1 | 3152 | C | CA | Asn245fs | 3.08 | NS5 | 9658 | T | A | Ser697Thr | 0.97 |
| NS1 | 3206 | C | G | Thr262Arg | 1.36 | NS5 | 9683 | A | T | Gln705Leu | 1.32 |
| NS1 | 3284 | C | G | Thr288Ser | 1.48 | NS5 | 9689 | C | G | Pro707Arg | 0.90 |
| NS1 | 3299 | A | T | Asn293Ile | 1.23 | NS5 | 9692 | T | A | Phe708Tyr | 0.79 |
| NS1 | 3309 | C | A | - | 1.57 | NS5 | 9696 | T | A | Cys709* | 1.25 |
| NS1 | 3323 | C | G | Thr301Ser | 1.11 | NS5 | 9702 | C | G | His711Gln | 1.80 |
| NS1 | 3345 | A | T | - | 0.79 | NS5 | 9742 | G | C | Val725Leu | 1.19 |
| NS1 | 3350 | A | T | Glu310Val | 0.83 | NS5 | 9753 | C | G | Cys728Trp | 0.89 |
| NS1 | 3375 | A | T | Leu318Phe | 0.87 | NS5 | 9757 | A | T | Asn730Tyr | 1.36 |
| NS1 | 3385 | A | T | Arg322* | 0.87 | NS5 | 9767 | A | C | Glu733Ala | 0.81 |
| NS1 | 3475 | G | C | Ala352Pro | 1.35 | NS5 | 9773 | T | A | Ile735Asn | 0.92 |
| NS2A | 3540 | A | T | Glu21Asp | 1.38 | NS5 | 9775 | G | C | Gly736Arg | 1.07 |

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|---|---|---|---|---|---|
| NS2A | 3611 | C | G | Thr45Arg | 1.15 |
| NS2A | 3617 | A | T | Asn47Ile | 0.87 |
| NS2A | 3633 | C | G | Asp52Glu | 1.33 |
| NS2A | 3655 | G | C | Val60Leu | 0.91 |
| NS2A | 3689 | G | C | Gly71Ala | 0.92 |
| NS2A | 3763 | C | G | Leu96Val | 1.24 |
| NS2A | 3787 | G | C | Ala104Pro | 0.91 |
| NS2A | 3793 | A | T | Ile106Leu | 1.14 |
| NS2A | 3848 | C | G | Thr124Ser | 1.25 |
| NS2A | 3889 | A | T | Arg138* | 0.72 |
| NS2A | 3918 | G | C | - | 0.87 |
| NS2A | 3924 | C | G | Ile149Met | 0.88 |
| NS2A | 3940 | G | T | Val155Phe | 0.93 |
| NS2A | 3943 | C | G | Pro156Ala | 0.99 |
| NS2A | 4013 | C | G | Pro179Arg | 1.35 |
| NS2A | 4061 | C | G | Ala195Gly | 1.16 |
| NS2B | 4205 | A | T | Asp25Val | 0.69 |
| NS2B | 4216 | A | T | Thr29Ser | 0.99 |
| NS2B | 4339 | A | T | Ser70Cys | 1.13 |
| NS2B | 4365 | A | T | - | 0.95 |
| NS2B | 4370 | A | T | Glu80Val | 0.83 |
| NS2B | 4431 | A | T | Arg100Ser | 0.96 |
| NS2B | 4452 | A | T | - | 0.90 |
| NS3 | 4528 | G | T | Val3Leu | 0.83 |
| NS3 | 4530 | A | T | - | 1.19 |
| NS3 | 4583 | G | C | Gly21Ala | 1.05 |
| NS5 | 9781 | G | C | Ala738Pro | 1.23 |
| NS5 | 9815 | A | T | Arg749Leu | 1.53 |
| NS5 | 9839 | C | G | Ser757Cys | 1.04 |
| NS5 | 9851 | T | A | Met761Lys | 1.07 |
| NS5 | 9858 | C | G | Ser763Arg | 1.18 |
| NS5 | 9876 | A | T | Arg769Ser | 1.17 |
| NS5 | 9877 | C | G | Arg770Gly | 1.06 |
| NS5 | 9882 | C | G | Asp771Glu | 1.16 |
| NS5 | 9939 | A | T | - | 1.01 |
| NS5 | 10015 | G | C | Val816Leu | 1.27 |
| NS5 | 10066 | T | A | Trp833Arg | 0.91 |
| NS5 | 10120 | A | T | Ile851Phe | 1.12 |
| NS5 | 10135 | A | T | Arg856Trp | 0.95 |
| NS5 | 10142 | C | G | Thr858Ser | 1.18 |
| NS5 | 10167 | A | T | - | 0.84 |
| NS5 | 10206 | C | G | Tyr879* | 1.51 |
| 3UTR | 10357 | G | C | - | 0.92 |
| 3UTR | 10410 | A | T | - | 1.07 |
| 3UTR | 10422 | G | C | - | 1.64 |
| 3UTR | 10436 | G | C | - | 1.38 |
| 3UTR | 10443 | T | TA | - | 0.60 |
| 3UTR | 10487 | C | G | - | 1.18 |
| 3UTR | 10578 | A | T | - | 1.60 |
| 3UTR | 10583 | C | G | - | 2.49 |
| 3UTR | 10587 | A | T | - | 1.65 |
| 3UTR | 10666 | A | T | - | 3.32 |

| **142** | | | | | |
|---|---|---|---|---|---|
| DF | | | | | |
| Primary | | | | | |
| 1.61E+03 | | | | | |
| 5 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| prM/M | 545 | T | A | Leu36His | 2.79 |
| E | 956 | C | G | Ser7* | 2.60 |
| E | 1379 | A | T | Glu148Val | 3.30 |
| NS1 | 2505 | G | C | Trp28Cys | 3.13 |
| NS1 | 3152 | C | CA | Asn245fs | 3.33 |
| NS2A | 3832 | A | T | Thr119Ser | 3.93 |
| NS3 | 4845 | T | C | - | 19.51 |
| NS3 | 5915 | C | CA | Asn467fs | 7.87 |
| NS4A-2K | 6789 | C | G | - | 3.79 |
| NS5 | 7748 | CA | C | Lys61fs | 3.39 |
| NS5 | 9654 | AC | A | Pro696fs | 1.67 |
| **143** | | | | | |
| SD | | | | | |
| Secondary | | | | | |
| 2.81E+02 | | | | | |
| ND | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| E | 2007 | C | T | - | 18.18 |
| E | 2010 | G | A | - | 18.18 |
| NS1 | 3114 | C | A | - | 25.00 |
| NS1 | 3129 | A | G | - | 23.53 |
| NS2A | 3663 | C | T | - | 16.67 |
| NS2A | 3876 | C | T | - | 33.33 |
| NS3 | 4562 | G | A | Gly14Glu | 27.27 |
| NS3 | 5915 | C | CA | Asn467fs | 16.67 |
| E | 1853 | T | A | Phe306Tyr | 1.70 |
| NS1 | 2495 | T | A | Val25Glu | 1.04 |
| NS1 | 2511 | G | C | Glu30Asp | 1.51 |
| NS1 | 2632 | A | T | Ile71Leu | 1.16 |
| NS1 | 2745 | C | T | - | 5.23 |
| NS1 | 2751 | G | C | Glu110Asp | 1.68 |
| NS1 | 2786 | A | T | Lys122Ile | 1.54 |
| NS1 | 2807 | A | T | His129Leu | 1.13 |
| NS1 | 3268 | A | T | Thr283Ser | 1.76 |
| NS1 | 3375 | A | T | Leu318Phe | 1.18 |
| NS2A | 3787 | G | C | Ala104Pro | 1.45 |
| NS2A | 4013 | C | G | Pro179Arg | 1.42 |
| NS2A | 4074 | A | T | Lys199Asn | 1.28 |
| NS2B | 4437 | A | T | - | 1.24 |
| NS2B | 4488 | A | T | - | 2.03 |
| NS3 | 4577 | A | T | Glu19Val | 1.64 |
| NS3 | 4681 | C | G | Arg54Gly | 1.40 |
| NS3 | 4747 | C | G | Leu76Val | 1.55 |
| NS3 | 5026 | G | GA | Ser171fs | 2.59 |
| NS3 | 5148 | A | T | Arg209Ser | 1.08 |
| NS3 | 5192 | C | G | Thr224Ser | 1.66 |
| NS3 | 5233 | C | A | Leu238Ile | 1.46 |
| NS3 | 5330 | T | A | Leu270Gln | 1.97 |
| NS3 | 5485 | A | T | Arg322* | 1.82 |
| NS3 | 5630 | A | T | Asp370Val | 2.16 |
| NS3 | 5915 | C | CA | Asn467fs | 13.32 |
| NS3 | 5931 | C | G | Asp470Glu | 1.46 |
| NS3 | 5977 | G | C | Ala486Pro | 2.38 |
| NS3 | 6131 | T | A | Met537Lys | 1.49 |
| NS3 | 6330 | T | A | Asp603Glu | 1.66 |
| NS3 | 6335 | T | A | Leu605Gln | 1.91 |

| | | | | | |
|---|---|---|---|---|---|
| NS5 | 9034 | C | T | - | 22.22 |
| NS5 | 9840 | Y | C | - | 40.00 |
| NS5 | 9909 | T | C | - | 40.00 |
| **144** | | | | | |
| WS | | | | | |
| Primary | | | | | |
| 7.60E+02 | | | | | |
| ND | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 187 | A | C | Lys31Gln | 4.13 |
| E | 1285 | G | C | Ala117Pro | 5.13 |
| E | 1798 | A | T | Arg288* | 4.30 |
| NS1 | 3084 | A | AT | Asn222fs | 2.94 |
| NS2A | 3689 | G | C | Gly71Ala | 4.85 |
| NS2B | 4211 | C | G | Pro27Arg | 3.70 |
| NS3 | 5531 | G | T | Arg337Ile | 4.80 |
| NS3 | 5915 | C | CA | Asn467fs | 8.51 |
| **145** | | | | | |
| DF | | | | | |
| Secondary | | | | | |
| 2.47E+02 | | | | | |
| 3 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 70 | G | GT | | 2.09 |
| 5UTR | 95 | T | A | - | 2.17 |
| C | 241 | G | C | Ala49Pro | 1.68 |
| C | 418 | T | C | - | 5.19 |
| prM/M | 703 | G | GA | Arg91fs | 1.93 |
| E | 1067 | A | T | Glu44Val | 1.98 |
| E | 1071 | GAT | G | Ile46fs | 0.77 |
| E | 1224 | G | C | Met96Ile | 2.23 |
| E | 1789 | T | A | Cys285Ser | 1.55 |
| NS4A | 6575 | G | T | Gly67Val | 2.63 |
| NS4A | 6616 | A | T | Met81Leu | 1.11 |
| NS4A | 6651 | C | G | Ser92Arg | 1.28 |
| NS4A | 6652 | A | T | Ile93Phe | 1.57 |
| NS4A | 6653 | T | G | Ile93Ser | 1.15 |
| NS4A | 6682 | C | A | His103Asn | 1.55 |
| NS4A | 6682 | C | G | His103Asp | 1.86 |
| NS4A | 6711 | G | GT | Leu115fs | 1.57 |
| NS4B | 6923 | G | C | Arg33Pro | 1.94 |
| NS4B | 7173 | A | T | - | 1.25 |
| NS4B | 7282 | A | T | Ile153Phe | 1.83 |
| NS4B | 7433 | C | G | Thr203Arg | 1.94 |
| NS5 | 7786 | C | G | Pro73Ala | 2.30 |
| NS5 | 8012 | G | C | Gly148Ala | 1.81 |
| NS5 | 8392 | G | C | Asp275His | 1.26 |
| NS5 | 8407 | A | T | Arg280* | 1.46 |
| NS5 | 8586 | A | T | - | 3.01 |
| NS5 | 8982 | C | G | Ser471Arg | 1.74 |
| NS5 | 8988 | C | G | - | 2.24 |
| NS5 | 9112 | C | G | Leu515Val | 1.95 |
| NS5 | 9369 | T | C | - | 14.61 |
| NS5 | 9481 | A | T | Thr638Ser | 2.75 |
| NS5 | 9569 | T | A | Val667Glu | 1.67 |
| NS5 | 9662 | G | C | Arg698Thr | 1.51 |
| NS5 | 9701 | A | T | His711Leu | 1.13 |
| NS5 | 9840 | C | G | - | 1.54 |
| NS5 | 9846 | C | G | - | 2.01 |
| NS5 | 9869 | T | A | Phe767Tyr | 1.61 |
| NS5 | 9880 | G | T | Asp771Tyr | 1.70 |
| NS5 | 9965 | C | G | Ala799Gly | 1.98 |
| 3UTR | 10578 | A | T | - | 2.70 |
| 3UTR | 10611 | C | CA | - | 2.56 |

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|---|---|---|---|---|---|
| **146** | | | | | |
| DF | | | | | |
| Secondary | | | | | |
| 2.29E+05 | | | | | |
| 2 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 273 | C | T | - | 0.62 |
| C | 413 | T | C | Ile106Thr | 2.88 |
| E | 2015 | A | G | Glu360Gly | 0.70 |
| E | 2256 | C | T | - | 1.10 |
| NS1 | 3212 | C | T | - | 0.54 |
| NS2A | 3495 | C | T | - | 0.81 |
| NS2A | 3768 | C | T | - | 0.76 |
| NS3 | 5034 | C | T | - | 0.53 |
| NS3 | 5649 | G | A | - | 2.47 |
| NS3 | 5915 | C | CA | Asn467fs | 14.06 |
| NS4A | 6732 | C | T | - | 5.74 |
| NS4A-2K | 6801 | C | T | - | 1.65 |
| NS4B | 7306 | G | A | Asp161Asn | 0.51 |
| NS5 | 8889 | T | C | - | 0.80 |
| 3UTR | 10611 | C | CA | - | 1.61 |
| **147** | | | | | |
| WS | | | | | |
| Secondary | | | | | |
| 1.73E+03 | | | | | |
| NS1 | 2489 | A | T | Asp23Val | 1.54 |
| NS1 | 2495 | T | A | Val25Glu | 2.12 |
| NS1 | 2517 | T | A | Tyr32* | 1.45 |
| NS1 | 2601 | A | T | - | 1.57 |
| NS1 | 2632 | A | T | Ile71Leu | 1.87 |
| NS1 | 2643 | A | T | Glu74Asp | 2.57 |
| NS1 | 2769 | A | T | Lys116Asn | 1.23 |
| NS1 | 3152 | C | CA | Asn245fs | 2.48 |
| NS1 | 3193 | C | G | Pro258Ala | 2.03 |
| NS1 | 3324 | C | A | - | 1.60 |
| NS2A | 3510 | A | T | - | 2.36 |
| NS2A | 3699 | T | A | Tyr74* | 0.80 |
| NS2A | 3974 | A | T | Lys166Ile | 1.18 |
| NS2A | 3979 | A | T | Ser168Cys | 1.32 |
| NS2A | 4128 | A | T | Lys217Asn | 2.10 |
| NS2B | 4205 | A | T | Asp25Val | 0.84 |
| NS2B | 4222 | C | G | Pro31Ala | 1.26 |
| NS2B | 4365 | A | T | - | 1.34 |
| NS2B | 4388 | T | TA | Asn88fs | 0.93 |
| NS2B | 4462 | C | G | Pro111Ala | 2.05 |
| NS2B | 4510 | A | T | Lys127* | 1.65 |
| NS3 | 4589 | A | T | Tyr23Phe | 1.95 |
| NS3 | 4656 | A | T | - | 1.23 |
| NS3 | 4993 | A | T | Ser158Cys | 1.41 |
| NS3 | 5143 | G | C | Val208Leu | 1.60 |

| 2 | | | | | |
|---|---|---|---|---|---|
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| E | 1341 | G | A | - | 0.80 |
| NS2A | 4099 | C | T | - | 1.94 |
| NS3 | 5915 | C | CA | Asn466fs | 13.50 |
| 3UTR | 10368 | TA | T | - | 0.58 |
| 3UTR | 10540 | CG | C | - | 0.66 |
| 3UTR | 10599 | AC | A | - | 1.55 |
| 3UTR | 10611 | C | CA | - | 1.19 |
| **148** | | | | | |
| WS | | | | | |
| Secondary | | | | | |
| 1.06E+02 | | | | | |
| 1 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 135 | C | T | - | 2.12 |
| C | 198 | A | T | - | 2.93 |
| C | 203 | G | C | Gly36Ala | 2.10 |
| C | 333 | T | C | - | 1.15 |
| C | 363 | A | T | - | 1.85 |
| prM/M | 682 | A | C | Thr82Pro | 4.35 |
| prM/M | 731 | A | T | His98Leu | 1.51 |
| prM/M | 878 | C | G | Thr147Arg | 1.34 |
| E | 940 | C | G | Arg2Gly | 1.47 |
| E | 950 | G | C | Gly5Ala | 1.70 |
| E | 1003 | A | T | Ile23Leu | 1.55 |
| E | 1005 | C | A | - | 2.05 |
| E | 1040 | C | CA | Asn37fs | 3.26 |
| E | 1067 | A | T | Glu44Val | 2.14 |
| E | 1102 | C | G | Leu56Val | 1.18 |
| E | 1111 | T | A | Tyr59Asn | 1.08 |
| E | 1112 | A | T | Tyr59Phe | 1.77 |
| E | 1373 | G | C | Gly146Ala | 1.40 |
| E | 1447 | A | T | Thr171Ser | 1.69 |
| E | 1590 | G | C | Leu218Phe | 1.12 |
| E | 1637 | A | T | Lys234Ile | 1.88 |
| E | 1686 | C | G | - | 1.39 |
| E | 1695 | A | G | - | 2.30 |
| E | 1702 | C | G | Gln256Glu | 1.56 |
| E | 1735 | G | C | Ala267Pro | 1.71 |
| E | 1821 | A | T | Lys295Asn | 1.35 |
| E | 1847 | G | C | Gly304Ala | 1.83 |
| E | 1891 | A | T | Thr319Ser | 1.69 |
| E | 2058 | A | T | - | 1.47 |
| NS3 | 5186 | C | G | Ala222Gly | 1.98 |
| NS3 | 5289 | C | G | Ile256Met | 2.08 |
| NS3 | 5318 | C | G | Thr266Ser | 1.55 |
| NS3 | 5330 | T | A | Leu270Gln | 1.45 |
| NS3 | 5424 | A | T | - | 1.94 |
| NS3 | 5485 | A | T | Arg322* | 2.88 |
| NS3 | 5788 | A | T | Ile423Leu | 1.44 |
| NS3 | 5826 | A | T | - | 2.14 |
| NS3 | 5828 | A | C | Asp436Ala | 1.58 |
| NS3 | 5859 | T | C | - | 2.01 |
| NS3 | 5870 | C | G | Thr450Ser | 2.24 |
| NS3 | 5915 | C | CA | Asn467fs | 9.32 |
| NS3 | 5945 | TG | T | Glu477fs | 0.49 |
| NS3 | 6335 | T | A | Leu605Gln | 2.62 |
| NS4A | 6481 | G | C | Ala36Pro | 1.32 |
| NS4A | 6510 | C | A | - | 1.99 |
| NS4A | 6575 | G | T | Gly67Val | 2.40 |
| NS4A | 6651 | C | G | Ser92Arg | 1.35 |
| NS4B | 6982 | C | T | Arg53* | 1.77 |
| NS4B | 7051 | A | T | Met76Leu | 1.55 |
| NS4B | 7288 | C | G | Leu155Val | 1.50 |
| NS4B | 7314 | A | T | Lys163Asn | 1.51 |
| NS4B | 7439 | G | C | Trp205Ser | 1.82 |
| NS5 | 8004 | T | A | Cys145* | 2.01 |
| NS5 | 8019 | G | C | - | 1.46 |
| NS5 | 8162 | A | T | Gln198Leu | 1.48 |
| NS5 | 8181 | C | G | - | 1.63 |
| NS5 | 8220 | A | T | Glu217Asp | 1.80 |
| NS5 | 8388 | T | A | Asn273Lys | 1.38 |
| NS5 | 8752 | A | T | Thr395Ser | 1.59 |
| NS5 | 8847 | T | A | - | 1.38 |
| NS5 | 8932 | A | T | Met455Leu | 1.40 |
| NS5 | 8985 | A | T | Arg472Ser | 1.81 |
| NS5 | 9014 | G | C | Arg482Pro | 1.35 |
| NS5 | 9082 | A | T | Ser505Cys | 1.69 |
| NS5 | 9477 | C | T | - | 2.05 |
| NS5 | 9599 | C | G | Ala677Gly | 1.27 |
| NS5 | 9658 | T | A | Ser697Thr | 1.37 |
| NS5 | 9839 | C | G | Ser757Cys | 1.21 |
| NS5 | 10005 | C | G | - | 2.17 |
| NS5 | 10009 | A | T | Asn814Tyr | 1.97 |
| NS5 | 10167 | A | T | - | 2.53 |
| 3UTR | 10340 | T | A | - | 1.99 |
| 3UTR | 10583 | C | G | - | 3.52 |

| **149** | | | | | |
|---|---|---|---|---|---|
| SD | | | | | |
| Primary | | | | | |
| 4.94E+05 | | | | | |
| 5 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| E | 1040 | C | CA | Asn37fs | 5.97 |
| E | 1748 | A | T | Gln271Leu | 0.88 |
| E | 1753 | T | A | Ser273Thr | 0.60 |
| E | 2065 | T | A | Tyr377His | 0.63 |
| NS1 | 2538 | T | C | - | 0.47 |
| NS3 | 4829 | G | A | Gly103Glu | 0.79 |
| NS3 | 5361 | G | A | - | 0.47 |
| NS2A | 4122 | C | G | Ser215Arg | 1.61 |
| NS2B | 4199 | A | T | Lys23Ile | 1.46 |
| NS2B | 4211 | C | G | Pro27Arg | 1.80 |
| NS2B | 4216 | A | T | Thr29Ser | 1.29 |
| NS2B | 4333 | T | A | Ser68Thr | 1.34 |
| NS2B | 4339 | A | T | Ser70Cys | 1.55 |
| NS2B | 4355 | C | G | Ser75* | 1.16 |
| NS3 | 4649 | A | T | Glu43Val | 1.51 |
| NS3 | 4708 | A | T | Lys63* | 1.31 |
| NS3 | 4743 | G | A | - | 11.29 |
| NS3 | 4795 | G | C | Gly92Arg | 1.84 |
| NS3 | 4848 | C | G | - | 1.67 |
| NS3 | 4954 | A | T | Lys145* | 1.71 |

| | | | | | |
|---|---|---|---|---|---|
| NS3 | 5772 | C | T | - | 1.64 |
| NS3 | 5915 | C | CA | Asn467fs | 15.06 |
| NS3 | 6171 | G | A | - | 36.07 |
| NS4B | 6844 | G | A | Glu7Lys | 0.77 |
| NS5 | 8727 | G | A | - | 32.48 |
| NS5 | 8916 | A | G | - | 0.96 |
| NS5 | 9512 | C | A | Ala648Glu | 3.30 |
| 3UTR | 10711 | A | T | - | 30.77 |
| **151** | | | | | |
| DF | | | | | |
| Secondary | | | | | |
| 3.82E+03 | | | | | |
| 2 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 70 | G | GT | - | 1.89 |
| C | 141 | G | C | Met15Ile | 1.92 |
| prM/M | 462 | A | T | - | 1.10 |
| prM/M | 703 | G | GA | Arg91fs | 1.04 |
| prM/M | 721 | C | G | Leu95Val | 2.04 |
| prM/M | 727 | C | G | Pro97Ala | 1.48 |
| E | 964 | G | C | Asp10His | 1.53 |
| E | 1040 | C | CA | Asn37fs | 2.83 |
| E | 1067 | A | T | Glu44Val | 2.20 |
| E | 1109 | A | T | Lys58Met | 1.32 |
| E | 1177 | A | T | Ser81Cys | 1.47 |
| E | 1218 | C | G | His94Gln | 1.39 |
| E | 1371 | A | T | - | 0.96 |
| E | 1564 | A | T | Arg210Trp | 1.23 |
| E | 1621 | T | A | Ser229Thr | 1.36 |
| E | 1729 | A | T | Thr265Ser | 1.27 |
| E | 2021 | A | T | Asp362Val | 1.98 |
| E | 2074 | G | C | Val380Leu | 1.77 |
| E | 2120 | G | C | Gly395Ala | 1.90 |
| E | 2301 | G | C | Met455Ile | 1.41 |
| NS1 | 2495 | T | A | Val25Glu | 1.02 |
| NS1 | 2511 | G | C | Glu30Asp | 1.40 |
| NS1 | 2601 | A | T | - | 1.26 |
| NS1 | 2693 | G | C | Gly91Ala | 1.47 |
| NS1 | 2699 | T | A | Ile93Asn | 1.31 |
| NS1 | 2741 | A | T | Gln107Leu | 1.23 |
| NS1 | 2745 | C | G | - | 1.60 |
| NS1 | 2791 | C | G | Leu124Val | 1.13 |
| NS1 | 3152 | C | CA | Asn245fs | 3.39 |
| NS1 | 3187 | T | A | Tyr256Asn | 1.71 |
| NS1 | 3233 | G | C | Gly271Ala | 1.93 |
| NS1 | 3375 | A | T | Leu318Phe | 1.28 |
| NS2A | 3689 | G | C | Gly71Ala | 1.02 |
| NS2A | 3834 | C | A | - | 1.23 |
| NS2A | 3849 | T | A | - | 0.93 |
| NS2A | 4064 | T | A | Leu196* | 1.36 |
| NS3 | 5143 | G | C | Val208Leu | 1.16 |
| NS3 | 5314 | T | A | Phe265Ile | 1.29 |
| NS3 | 5473 | C | G | Pro318Ala | 1.12 |
| NS3 | 5694 | C | G | Asp391Glu | 1.51 |
| NS3 | 5700 | G | C | Glu393Asp | 1.85 |
| NS3 | 5718 | C | G | - | 1.90 |
| NS3 | 5724 | T | A | Asp401Glu | 1.70 |
| NS3 | 5815 | G | C | Val432Leu | 1.61 |
| NS3 | 5832 | C | G | - | 2.56 |
| NS3 | 5915 | C | CA | Asn467fs | 8.57 |
| NS3 | 5931 | C | G | Asp470Glu | 2.19 |
| NS3 | 5975 | G | T | Cys485Phe | 1.44 |
| NS3 | 6330 | T | A | Asp603Glu | 2.14 |
| NS4A | 6456 | C | G | Asn27Lys | 1.53 |
| NS4A | 6510 | C | A | - | 1.68 |
| NS4A | 6606 | T | A | - | 1.36 |
| NS4A | 6651 | C | G | Ser92Arg | 1.92 |
| NS4A | 6657 | C | G | - | 1.96 |
| NS4A | 6674 | T | A | Ile100Lys | 1.31 |
| NS4A | 6711 | G | GT | Leu115fs | 0.88 |
| NS4B | 6955 | G | C | Ala44Pro | 1.45 |
| NS4B | 6979 | T | A | Leu52Met | 1.53 |
| NS4B | 6983 | G | T | Arg53Ile | 1.46 |
| NS4B | 7332 | A | T | - | 1.70 |
| NS4B | 7335 | A | T | Gln170His | 1.89 |
| NS4B | 7391 | C | G | Ala189Gly | 2.11 |
| NS5 | 7673 | A | T | Glu35Val | 1.88 |
| NS5 | 7835 | A | T | Tyr89Phe | 2.30 |
| NS5 | 8007 | C | G | Asp146Glu | 1.67 |
| NS5 | 8137 | G | C | Val190Leu | 1.66 |
| NS5 | 8178 | A | T | - | 1.15 |
| NS5 | 8238 | T | A | Asn223Lys | 1.14 |
| NS5 | 8306 | A | T | Lys246Ile | 1.53 |
| NS5 | 8322 | C | G | - | 1.30 |
| NS5 | 8454 | C | G | Asp295Glu | 1.38 |
| NS5 | 8520 | A | T | - | 2.04 |
| NS5 | 8570 | C | T | Ala334Val | 8.12 |
| NS5 | 8793 | C | G | - | 2.40 |
| NS5 | 9112 | C | G | Leu515Val | 2.36 |
| NS5 | 9121 | A | T | Ile518Phe | 2.09 |
| NS5 | 9187 | A | T | Thr540Ser | 2.81 |
| NS5 | 9413 | T | A | Met615Lys | 1.57 |
| NS5 | 9587 | G | T | Arg673Met | 1.27 |
| NS5 | 9652 | G | C | Glu695Gln | 1.80 |
| NS5 | 9736 | G | C | Val723Leu | 1.07 |
| NS5 | 9742 | G | C | Val725Leu | 1.54 |
| NS5 | 10167 | A | T | - | 1.81 |
| NS5 | 10212 | C | G | Asp881Glu | 1.35 |
| 3UTR | 10314 | A | T | - | 2.08 |
| 3UTR | 10611 | C | CAAAA | - | 13.04 |

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|---|---|---|---|---|---|
| **153** | | | | | |
| WS | | | | | |
| Primary | | | | | |
| 1.06E+02 | | | | | |
| 12 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 70 | G | GT | - | 1.75 |
| NS5 | 8137 | G | A | Val190Ile | 1.07 |
| NS5 | 8693 | A | G | Lys375Arg | 1.00 |
| NS5 | 9209 | T | TA | Asn549fs | 0.51 |
| NS5 | 9449 | G | A | Gly627Glu | 0.85 |
| 3UTR | 10331 | G | A | - | 0.63 |
| **155** | | | | | |
| DF | | | | | |

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|---|---|---|---|---|---|
| C | 187 | A | C | Lys31Gln | 2.72 |
| C | 235 | T | A | Phe47Ile | 2.27 |
| C | 355 | G | T | Glu87* | 2.06 |
| C | 436 | GC | G | Ala114fs | 0.53 |
| prM/M | 470 | A | T | His11Leu | 1.85 |
| prM/M | 703 | G | GA | Arg91fs | 2.35 |
| prM/M | 884 | T | A | Phe149Tyr | 2.13 |
| E | 1479 | C | G | - | 1.93 |
| E | 1587 | GT | G | Leu218fs | 0.54 |
| E | 1631 | T | A | Ile232Lys | 2.39 |
| E | 1779 | A | T | - | 2.07 |
| E | 1957 | G | C | Asp341His | 2.14 |
| E | 2347 | C | G | Arg471Gly | 1.81 |
| NS1 | 2511 | G | C | Glu30Asp | 2.20 |
| NS1 | 3152 | C | CA | Asn245fs | 2.66 |
| NS1 | 3279 | G | T | - | 2.02 |
| NS2A | 3513 | C | G | - | 2.68 |
| NS2A | 3699 | T | A | Tyr74* | 1.52 |
| NS2A | 3967 | G | C | Ala164Pro | 1.69 |
| NS2B | 4355 | C | G | Ser75* | 2.35 |
| NS2B | 4388 | T | TA | Asn88fs | 2.03 |
| NS2B | 4412 | C | G | Thr94Arg | 2.30 |
| NS3 | 4829 | G | GA | Asn105fs | 1.89 |
| NS3 | 5143 | G | C | Val208Leu | 2.06 |
| NS3 | 5192 | C | G | Thr224Ser | 2.26 |
| NS3 | 5224 | C | G | Leu235Val | 1.35 |
| NS3 | 5447 | C | T | Ala309Val | 2.21 |
| NS3 | 5592 | G | C | Lys357Asn | 1.69 |
| NS3 | 5915 | C | CA | Asn467fs | 9.23 |
| NS3 | 6244 | GA | G | Asn576fs | 0.84 |
| NS3 | 6319 | A | T | Ile600Phe | 1.87 |
| NS3 | 6330 | T | A | Asp603Glu | 2.70 |
| NS4A | 6616 | A | T | Met81Leu | 2.13 |
| NS4B | 6928 | G | C | Ala35Pro | 2.34 |
| NS4B | 7310 | C | A | Pro162Gln | 1.80 |
| NS5 | 8071 | G | C | Val168Leu | 2.97 |
| NS5 | 8398 | A | T | Ile277Phe | 3.36 |
| NS5 | 8919 | T | A | Cys450* | 2.08 |
| NS5 | 9807 | G | T | Trp746Cys | 2.21 |
| NS5 | 9965 | C | G | Ala799Gly | 2.11 |
| NS5 | 9966 | C | G | - | 1.93 |
| NS5 | 10182 | A | T | Arg871Ser | 2.22 |
| 3UTR | 10583 | C | G | - | 5.23 |

**154**

DF
Secondary
1.56E+05
2

| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
|---|---|---|---|---|---|
| 5UTR | 70 | G | GT | - | 1.97 |
| E | 1385 | C | T | Ala150Val | 2.38 |
| NS1 | 2967 | A | G | - | 1.22 |
| NS3 | 4562 | G | A | Gly14Glu | 1.99 |
| NS3 | 5304 | T | G | Cys261Trp | 1.06 |
| NS3 | 5649 | G | A | - | 0.90 |
| NS3 | 5887 | C | T | Gln456* | 0.66 |
| NS3 | 5915 | C | CA | Asn467fs | 15.47 |
| NS3 | 6336 | G | A | - | 13.41 |
| NS4B | 6844 | G | A | Glu7Lys | 0.64 |

Secondary
1.66E+05
2

| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
|---|---|---|---|---|---|
| E | 1316 | GA | G | Val128fs | 0.50 |
| E | 1748 | A | T | Gln271Leu | 0.58 |
| NS1 | 3069 | C | T | - | 1.30 |
| NS2B | 4434 | G | A | - | 2.81 |
| NS3 | 5304 | T | G | Cys261Trp | 1.29 |
| NS3 | 5424 | A | T | - | 1.30 |
| NS3 | 5915 | C | CA | Asn467fs | 12.70 |
| NS5 | 7842 | TG | T | Gly92fs | 0.67 |
| NS5 | 8204 | G | T | Arg212Leu | 0.50 |
| 3UTR | 10611 | C | CA | - | 1.44 |

**156**

WS
Primary
4.68E+00
3

| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
|---|---|---|---|---|---|
| C | 431 | T | C | Val112Ala | 15.00 |
| prM/M | 523 | A | G | Asn29Asp | 35.29 |
| prM/M | 525 | C | T | - | 26.32 |
| E | 1116 | T | C | - | 11.54 |
| E | 1188 | A | G | - | 20.00 |
| E | 1545 | T | C | - | 22.22 |
| E | 1587 | G | A | - | 26.67 |
| E | 1626 | C | T | - | 18.18 |
| E | 1972 | T | C | Tyr346His | 23.08 |
| E | 2376 | G | A | - | 33.33 |
| NS1 | 2580 | C | T | - | 25.00 |
| NS1 | 2677 | T | C | - | 14.81 |
| NS1 | 2679 | A | G | - | 30.00 |
| NS1 | 2952 | A | T | - | 18.18 |
| NS1 | 3459 | C | T | - | 25.00 |
| NS2A | 3498 | T | C | - | 36.36 |
| NS2A | 3663 | C | T | - | 20.00 |
| NS2B | 4249 | G | A | Val40Ile | 11.11 |
| NS2B | 4380 | C | T | - | 13.04 |
| NS2B | 4438 | C | T | - | 23.81 |
| NS3 | 4971 | C | T | - | 42.31 |
| NS3 | 5025 | C | T | - | 22.73 |
| NS3 | 5235 | T | C | - | 38.46 |
| NS3 | 5251 | AC | A | Pro245fs | 7.69 |
| NS3 | 5850 | G | A | - | 20.00 |
| NS3 | 5915 | C | CA | Asn467fs | 14.29 |
| NS4B | 6864 | C | T | - | 23.08 |
| NS4B | 6871 | G | A | Gly16Arg | 20.00 |
| NS4B | 7018 | T | C | - | 20.69 |
| NS4B | 7041 | T | C | - | 17.86 |
| NS4B | 7098 | T | C | - | 25.00 |
| NS4B | 7182 | C | T | - | 21.43 |
| NS4B | 7503 | G | A | - | 35.71 |
| NS5 | 8143 | G | GA | Met194fs | 11.76 |
| NS5 | 8475 | G | A | - | 33.33 |
| NS5 | 8859 | T | G | Ser430Arg | 30.77 |
| NS5 | 9399 | T | C | - | 27.27 |
| NS5 | 9534 | A | G | - | 16.67 |
| NS5 | 9900 | T | C | - | 17.65 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | NS5 | 10075 | A | G | Ile836Val | 33.33 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| **157** | | | | | | | | | | | |
| WS | | | | | | E | 2035 | A | T | Ile367Leu | 2.43 |
| Secondary | | | | | | E | 2414 | T | A | Val493Glu | 1.92 |
| 1.59E+02 | | | | | | NS1 | 2430 | T | A | - | 1.62 |
| 3 | | | | | | NS1 | 2495 | T | A | Val25Glu | 1.14 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS1 | 2505 | G | C | Trp28Cys | 1.45 |
| prM/M | 876 | G | C | - | 16.67 | NS1 | 2611 | G | C | Glu64Gln | 1.53 |
| E | 1084 | G | A | Ala50Thr | 15.38 | NS1 | 3152 | C | CA | Asn245fs | 2.92 |
| NS4B | 7008 | A | G | - | 23.08 | NS2A | 3688 | G | C | Gly71Arg | 1.46 |
| NS5 | 9912 | A | G | - | 20.00 | NS2A | 3693 | G | C | - | 1.52 |
| **158** | | | | | | NS2A | 3848 | C | G | Thr124Ser | 1.74 |
| DF | | | | | | NS2A | 3944 | C | G | Pro156Arg | 1.24 |
| Secondary | | | | | | NS2A | 4023 | A | T | Leu182Phe | 1.35 |
| 3.38E+04 | | | | | | NS2B | 4205 | A | T | Asp25Val | 1.06 |
| 1 | | | | | | NS2B | 4254 | T | A | Cys41* | 1.13 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS2B | 4382 | T | A | Met84Lys | 1.71 |
| 5UTR | 70 | G | GT | - | 2.98 | NS2B | 4413 | A | T | - | 2.15 |
| C | 111 | GA | G | Lys7fs | 2.92 | NS2B | 4458 | C | A | - | 2.07 |
| E | 1887 | T | C | - | 1.34 | NS3 | 4577 | A | T | Glu19Val | 1.96 |
| NS1 | 3053 | A | C | Lys211Thr | 0.92 | NS3 | 5010 | T | A | Ser163Arg | 1.35 |
| NS1 | 3152 | C | CA | Asn245fs | 5.19 | NS3 | 5026 | G | GA | Ser171fs | 3.28 |
| NS3 | 5304 | T | G | Cys261Trp | 1.41 | NS3 | 5136 | A | T | - | 1.71 |
| NS3 | 5847 | CT | C | Leu443fs | 0.47 | NS3 | 5218 | G | C | Glu233Gln | 1.74 |
| NS3 | 5886 | A | G | - | 0.76 | NS3 | 5231 | G | C | Gly237Ala | 1.42 |
| NS3 | 5915 | C | CA | Asn467fs | 16.76 | NS3 | 5239 | A | T | Ile240Leu | 1.31 |
| NS4A | 6480 | G | A | - | 1.48 | NS3 | 5487 | A | T | Arg322Ser | 1.78 |
| NS5 | 7842 | TG | T | Gly92fs | 0.47 | NS3 | 5493 | A | T | - | 1.38 |
| NS5 | 9489 | A | T | Glu640Asp | 0.80 | NS3 | 5516 | T | A | Ile332Asn | 1.69 |
| NS5 | 9705 | T | TC | Phe713fs | 0.46 | NS3 | 5575 | A | T | Thr352Ser | 1.64 |
| 3UTR | 10540 | CG | C | - | 0.53 | NS3 | 5915 | C | CA | Asn467fs | 14.33 |
| 3UTR | 10599 | AC | A | - | 2.15 | NS3 | 6319 | A | T | Ile600Phe | 2.40 |
| **159** | | | | | | NS3 | 6373 | A | T | Lys618* | 2.65 |
| DF | | | | | | NS4A | 6651 | C | G | Ser92Arg | 1.79 |
| Secondary | | | | | | NS4A | 6700 | A | T | Ile109Leu | 2.05 |
| 7.04E+03 | | | | | | NS4B | 6844 | G | GA | Glu7fs | 1.39 |
| 3 | | | | | | NS4B | 7111 | A | T | Ile96Phe | 2.05 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS4B | 7368 | A | T | - | 2.27 |
| 5UTR | 70 | G | GT | - | 2.13 | NS5 | 7673 | A | T | Glu35Val | 2.86 |
| prM/M | 695 | A | T | His86Leu | 2.76 | NS5 | 8047 | C | G | Arg160Gly | 1.93 |
| prM/M | 703 | G | GA | Arg91fs | 1.56 | NS5 | 8065 | A | T | Asn166Tyr | 1.68 |
| E | 956 | C | G | Ser7* | 1.82 | NS5 | 8409 | A | T | Arg280Ser | 1.78 |
| E | 1040 | C | CA | Asn37fs | 1.64 | NS5 | 8591 | T | A | Met341Lys | 3.88 |
| E | 1061 | A | T | Asp42Val | 1.86 | NS5 | 9112 | C | G | Leu515Val | 2.36 |
| E | 1067 | A | T | Glu44Val | 1.90 | NS5 | 9649 | T | A | Trp694Arg | 1.39 |
| E | 1111 | T | A | Tyr59Asn | 1.76 | NS5 | 9650 | G | C | Trp694Ser | 2.26 |
| E | 1112 | A | T | Tyr59Phe | 1.59 | NS5 | 9721 | A | T | Met718Leu | 1.00 |
| E | 1289 | T | A | Met118Lys | 1.56 | NS5 | 10005 | C | G | - | 1.54 |
| E | 1823 | G | C | Gly296Ala | 1.68 | NS5 | 10065 | A | T | - | 1.94 |
| E | 1839 | G | C | Met301Ile | 1.76 | 3UTR | 10552 | GT | G | - | 0.99 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| **160** | | | | | | NS3 | 4707 | A | G | - | 9.38 |
| DF | | | | | | NS3 | 4854 | G | A | - | 2.80 |
| Secondary | | | | | | NS3 | 4905 | A | G | - | 4.26 |
| 6.71E+04 | | | | | | NS3 | 4944 | T | C | - | 2.89 |
| 0 | | | | | | NS3 | 4974 | C | T | - | 6.35 |

| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
|---|---|---|---|---|---|
| C | 127 | A | T | Thr11Ser | 4.72 |
| C | 201 | C | T | - | 7.83 |
| C | 293 | TA | T | Lys67fs | 1.47 |
| C | 307 | A | G | Thr71Ala | 6.67 |
| C | 315 | A | G | - | 7.19 |
| C | 327 | T | C | - | 6.98 |
| C | 336 | C | T | - | 8.63 |
| prM/M | 445 | C | T | - | 3.19 |
| prM/M | 450 | T | C | - | 4.44 |
| prM/M | 508 | T | C | - | 2.88 |
| prM/M | 567 | A | G | - | 3.12 |
| prM/M | 703 | G | GA | Arg91fs | 8.82 |
| prM/M | 792 | A | G | - | 2.77 |
| prM/M | 798 | C | T | - | 3.81 |
| prM/M | 834 | C | T | - | 5.77 |
| prM/M | 837 | C | T | - | 5.96 |
| prM/M | 852 | A | G | - | 5.81 |
| prM/M | 856 | T | C | - | 5.56 |
| prM/M | 864 | T | C | - | 5.69 |
| prM/M | 873 | C | A | - | 6.15 |
| prM/M | 885 | C | T | - | 3.94 |
| prM/M | 894 | A | C | - | 6.35 |
| prM/M | 912 | A | G | - | 15.88 |
| prM/M | 921 | C | T | - | 5.38 |
| E | 954 | C | A | - | 9.25 |
| E | 1005 | A | C | - | 7.37 |
| E | 1040 | C | CA | Asn37fs | 2.29 |
| E | 1102 | T | C | - | 7.69 |
| E | 1293 | C | T | - | 3.49 |
| E | 1473 | T | C | - | 5.63 |
| E | 1590 | A | G | - | 4.63 |
| E | 1641 | A | G | - | 6.32 |
| E | 1860 | T | C | - | 14.10 |
| E | 1863 | A | G | - | 7.69 |
| E | 2223 | C | T | - | 1.72 |
| E | 2256 | C | T | - | 1.53 |
| E | 2307 | T | C | - | 3.48 |
| E | 2319 | A | C | - | 4.56 |
| E | 2328 | A | T | - | 2.24 |
| NS1 | 2434 | G | A | Val5Ile | 2.55 |
| NS1 | 2448 | G | A | - | 4.42 |
| NS1 | 2451 | C | T | - | 3.41 |
| NS1 | 2484 | C | T | - | 2.73 |
| NS1 | 2511 | A | G | - | 1.81 |
| NS1 | 2589 | G | A | - | 3.49 |
| NS1 | 2685 | T | C | - | 3.24 |
| NS1 | 2781 | A | G | - | 3.40 |
| NS1 | 2835 | T | C | - | 2.58 |
| NS1 | 2878 | A | C | Met153Leu | 8.67 |
| NS1 | 2898 | C | T | - | 5.39 |
| NS1 | 2961 | T | C | - | 7.47 |
| NS1 | 2976 | G | A | - | 6.10 |
| NS1 | 2991 | C | T | - | 5.08 |
| NS1 | 3129 | A | G | - | 7.97 |
| NS1 | 3152 | C | CA | Asn246fs | 8.20 |
| NS1 | 3234 | C | T | - | 4.68 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 1.27 |
| NS1 | 3300 | T | C | - | 1.80 |
| NS1 | 3330 | T | C | - | 6.42 |
| NS3 | 5026 | G | GA | Ser171fs | 4.07 |
| NS3 | 5190 | T | C | - | 4.31 |
| NS3 | 5277 | T | C | - | 5.81 |
| NS3 | 5283 | G | A | - | 4.60 |
| NS3 | 5304 | C | T | - | 7.57 |
| NS3 | 5613 | C | T | - | 6.86 |
| NS3 | 5730 | C | T | - | 4.00 |
| NS3 | 5802 | T | C | - | 5.79 |
| NS3 | 5915 | C | CA | Asn467fs | 8.82 |
| NS3 | 6009 | C | T | - | 9.74 |
| NS3 | 6033 | C | T | - | 4.40 |
| NS3 | 6069 | T | G | - | 7.97 |
| NS3 | 6094 | C | T | - | 7.64 |
| NS3 | 6099 | G | A | - | 6.55 |
| NS3 | 6220 | G | A | Val567Ile | 5.91 |
| NS3 | 6222 | C | T | - | 9.36 |
| NS3 | 6285 | G | GA | Lys590fs | 4.24 |
| NS3 | 6300 | T | C | - | 14.66 |
| NS3 | 6330 | C | T | - | 5.85 |
| NS4A | 6378 | C | T | - | 10.13 |
| NS4A | 6379 | C | T | - | 10.21 |
| NS4A | 6423 | C | T | - | 2.60 |
| NS4A | 6462 | C | T | - | 8.13 |
| NS4A | 6471 | T | C | - | 7.23 |
| NS4A | 6491 | G | A | Arg39Lys | 8.04 |
| NS4A | 6496 | C | T | His41Tyr | 8.72 |
| NS4A | 6500 | A | C | Asn42Thr | 8.61 |
| NS4A | 6528 | C | T | - | 13.04 |
| NS4A | 6547 | T | C | - | 13.33 |
| NS4A | 6556 | T | C | - | 10.90 |
| NS4A | 6562 | A | G | Thr63Ala | 10.59 |
| NS4A | 6585 | A | G | - | 10.03 |
| NS4A | 6633 | C | T | - | 11.06 |
| NS4A | 6654 | C | T | - | 11.96 |
| NS4A | 6681 | G | A | - | 10.87 |
| NS4A | 6711 | G | GT | Leu115fs | 2.53 |
| NS4B | 6841 | T | C | - | 5.16 |
| NS4B | 6864 | C | T | - | 3.06 |
| NS4B | 6879 | C | T | - | 5.53 |
| NS4B | 6922 | C | T | Arg33Cys | 1.93 |
| NS4B | 6929 | C | G | Ala35Gly | 1.35 |
| NS4B | 6948 | C | T | - | 4.50 |
| NS4B | 6951 | C | A | - | 1.46 |
| NS4B | 6982 | C | T | Arg53* | 1.72 |
| NS4B | 6993 | T | C | - | 27.27 |
| NS4B | 7041 | C | T | - | 5.49 |
| NS4B | 7059 | C | T | - | 8.05 |
| NS4B | 7062 | A | G | - | 6.42 |
| NS4B | 7083 | A | G | - | 8.66 |
| NS4B | 7092 | T | C | - | 6.74 |
| NS4B | 7248 | T | C | - | 14.29 |
| NS4B | 7314 | G | A | - | 5.98 |
| NS4B | 7356 | T | C | - | 2.86 |
| NS4B | 7383 | C | T | - | 3.66 |
| NS4B | 7428 | T | C | - | 3.74 |
| NS4B | 7551 | T | C | - | 4.38 |
| NS5 | 7627 | C | T | - | 41.67 |
| NS5 | 7656 | A | G | - | 12.22 |
| NS5 | 7911 | T | C | - | 4.72 |
| NS5 | 7959 | T | C | - | 5.60 |

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|---|---|---|---|---|---|
| NS1 | 3398 | A | G | Glu326Gly | 3.58 |
| NS1 | 3402 | C | T | - | 5.54 |
| NS1 | 3432 | C | T | - | 4.26 |
| NS1 | 3438 | G | A | - | 3.57 |
| NS1 | 3447 | G | A | - | 4.33 |
| NS1 | 3474 | G | A | - | 3.96 |
| NS2A | 3489 | G | A | - | 4.64 |
| NS2A | 3552 | T | C | - | 3.16 |
| NS2A | 3595 | T | C | Phe40Leu | 2.80 |
| NS2A | 3629 | A | G | Lys51Arg | 2.13 |
| NS2A | 3705 | C | T | - | 1.40 |
| NS2A | 3750 | A | G | - | 3.41 |
| NS2A | 3751 | CT | C | Leu92fs | 0.39 |
| NS2A | 3753 | T | C | - | 2.32 |
| NS2A | 3768 | T | C | - | 2.86 |
| NS2A | 3858 | A | G | - | 1.81 |
| NS2A | 3864 | A | G | - | 2.02 |
| NS2A | 3866 | G | C | Gly130Ala | 0.96 |
| NS2A | 3875 | T | C | Val133Ala | 2.23 |
| NS2A | 3876 | C | T | - | 2.01 |
| NS2A | 3879 | A | C | - | 2.63 |
| NS2A | 3921 | C | T | - | 3.28 |
| NS2A | 4011 | C | T | - | 3.69 |
| NS2A | 4034 | G | A | Arg186Gln | 4.49 |
| NS2A | 4036 | C | CA | Thr189fs | 0.64 |
| NS2A | 4038 | A | G | - | 10.82 |
| NS2A | 4063 | T | C | - | 3.10 |
| NS2A | 4069 | G | A | Val198Ile | 10.43 |
| NS2A | 4083 | T | C | - | 4.52 |
| NS2A | 4095 | T | C | - | 11.49 |
| NS2A | 4110 | C | T | - | 5.86 |
| NS2A | 4125 | G | A | - | 44.79 |
| NS2B | 4164 | T | C | - | 4.62 |
| NS2B | 4206 | C | T | - | 4.55 |
| NS2B | 4212 | C | T | - | 6.77 |
| NS2B | 4278 | T | C | - | 3.73 |
| NS2B | 4338 | G | A | - | 3.39 |
| NS2B | 4341 | C | T | - | 8.18 |
| NS2B | 4344 | T | C | - | 7.00 |
| NS2B | 4414 | T | C | - | 18.60 |
| NS2B | 4443 | G | T | - | 5.81 |
| NS2B | 4482 | A | G | - | 11.90 |
| NS2B | 4491 | G | A | - | 5.79 |
| NS2B | 4509 | T | G | - | 11.97 |
| NS5 | 7996 | C | T | - | 8.47 |
| NS5 | 7998 | G | T | Leu143Phe | 1.38 |
| NS5 | 8049 | G | A | - | 5.04 |
| NS5 | 8083 | T | C | - | 4.76 |
| NS5 | 8089 | A | G | Asn174Asp | 3.65 |
| NS5 | 8103 | T | C | - | 3.88 |
| NS5 | 8121 | C | T | - | 3.35 |
| NS5 | 8184 | G | A | - | 5.58 |
| NS5 | 8214 | G | A | - | 3.37 |
| NS5 | 8235 | T | C | - | 7.06 |
| NS5 | 8241 | C | T | - | 11.63 |
| NS5 | 8289 | T | C | - | 6.87 |
| NS5 | 8298 | T | C | - | 11.57 |
| NS5 | 8306 | A | G | Lys246Arg | 6.00 |
| NS5 | 8310 | C | T | - | 13.19 |
| NS5 | 8316 | A | G | - | 5.68 |
| NS5 | 8337 | T | G | - | 9.23 |
| NS5 | 8343 | A | C | - | 10.34 |
| NS5 | 8349 | T | C | - | 13.04 |
| NS5 | 8544 | T | C | - | 2.96 |
| NS5 | 8619 | G | A | - | 10.31 |
| NS5 | 8628 | T | C | - | 4.35 |
| NS5 | 8658 | C | T | - | 7.50 |
| NS5 | 8745 | G | A | - | 23.08 |
| NS5 | 8803 | A | G | Ile412Val | 10.13 |
| NS5 | 8891 | T | TA | His442fs | 2.31 |
| NS5 | 8967 | T | C | - | 5.50 |
| NS5 | 9066 | T | C | - | 5.31 |
| NS5 | 9399 | C | T | - | 11.90 |
| NS5 | 9424 | C | T | - | 7.04 |
| NS5 | 9501 | C | G | His644Gln | 6.60 |
| NS5 | 9576 | C | T | - | 7.11 |
| NS5 | 9628 | G | A | Val687Ile | 5.88 |
| NS5 | 9795 | A | G | - | 5.92 |
| NS5 | 9902 | C | G | Ala778Gly | 1.50 |
| NS5 | 9997 | C | A | Leu810Met | 11.11 |
| NS5 | 10000 | G | A | Ala811Thr | 11.76 |
| NS5 | 10083 | T | C | - | 2.86 |
| NS5 | 10155 | C | T | - | 2.43 |
| 3UTR | 10389 | CA | C | - | 25.80 |
| 3UTR | 10389 | C | CA | - | 9.70 |
| 3UTR | 10408 | C | T | - | 11.50 |
| 3UTR | 10444 | T | TA | - | 2.18 |
| 3UTR | 10607 | C | CA | - | 5.66 |

| **161** | | | | | |
|---|---|---|---|---|---|
| DF | | | | | |
| Primary | | | | | |
| 1.25E+04 | | | | | |
| 3 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 201 | C | T | - | 25.00 |
| C | 219 | T | C | - | 26.47 |
| C | 315 | A | G | - | 13.56 |
| C | 327 | T | C | - | 14.29 |
| C | 384 | T | C | - | 16.05 |
| C | 387 | A | G | - | 17.11 |
| prM/M | 445 | C | T | - | 11.11 |
| prM/M | 450 | C | T | - | 5.13 |
| NS3 | 4875 | C | T | - | 7.33 |
| NS3 | 4905 | A | G | - | 6.40 |
| NS3 | 4944 | T | C | - | 7.37 |
| NS3 | 4959 | C | T | - | 5.43 |
| NS3 | 5004 | T | C | - | 8.82 |
| NS3 | 5026 | G | GA | Ser171fs | 6.45 |
| NS3 | 5070 | T | C | - | 16.67 |
| NS3 | 5235 | T | C | - | 20.72 |
| NS3 | 5277 | T | C | - | 7.50 |
| NS3 | 5283 | G | A | - | 5.38 |
| NS3 | 5340 | T | C | - | 10.00 |
| NS3 | 5613 | C | T | - | 13.73 |
| NS3 | 5649 | A | G | - | 20.00 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| prM/M | 508 | T | C | - | 8.33 | NS3 | 5667 | G | A | - | 10.42 |
| prM/M | 567 | A | G | - | 7.77 | NS3 | 5730 | T | C | - | 13.58 |
| prM/M | 606 | C | T | - | 8.82 | NS3 | 5775 | A | G | - | 12.70 |
| prM/M | 651 | A | G | - | 20.69 | NS3 | 5793 | T | C | - | 15.00 |
| prM/M | 792 | A | G | - | 13.33 | NS3 | 5802 | T | C | - | 10.20 |
| prM/M | 798 | C | T | - | 12.12 | NS3 | 5823 | G | A | - | 33.93 |
| prM/M | 834 | C | T | - | 4.46 | NS3 | 5847 | T | C | - | 23.33 |
| prM/M | 837 | C | T | - | 3.47 | NS3 | 5979 | A | C | - | 8.00 |
| prM/M | 852 | A | G | - | 6.15 | NS3 | 6009 | C | T | - | 13.11 |
| prM/M | 855 | T | C | - | 12.21 | NS3 | 6069 | T | G | - | 28.13 |
| prM/M | 856 | T | C | - | 6.11 | NS3 | 6220 | G | A | Val567Ile | 17.31 |
| prM/M | 864 | T | C | - | 8.78 | NS3 | 6222 | C | T | - | 18.18 |
| prM/M | 873 | C | A | - | 9.59 | NS3 | 6285 | G | GA | Lys590fs | 5.88 |
| prM/M | 894 | A | C | - | 10.87 | NS3 | 6300 | T | C | - | 32.39 |
| prM/M | 921 | C | T | - | 8.54 | NS3 | 6330 | C | T | - | 16.67 |
| E | 954 | A | C | - | 2.94 | NS4A | 6378 | C | T | - | 11.81 |
| E | 1005 | A | C | - | 6.15 | NS4A | 6379 | C | T | - | 12.00 |
| E | 1040 | C | CA | Asn37fs | 5.71 | NS4A | 6462 | C | T | - | 13.04 |
| E | 1102 | T | C | - | 30.56 | NS4A | 6466 | T | C | - | 16.35 |
| E | 1287 | C | T | - | 23.08 | NS4A | 6471 | T | C | - | 10.19 |
| E | 1293 | C | T | - | 7.55 | NS4A | 6491 | G | A | Arg39Lys | 16.49 |
| E | 1353 | C | T | - | 10.68 | NS4A | 6496 | C | T | His41Tyr | 15.73 |
| E | 1368 | T | C | - | 22.08 | NS4A | 6500 | A | C | Asn42Thr | 11.32 |
| E | 1590 | A | G | - | 14.58 | NS4A | 6528 | C | T | - | 26.55 |
| E | 1641 | A | G | - | 11.19 | NS4A | 6547 | T | C | - | 27.17 |
| E | 1842 | T | C | - | 12.31 | NS4A | 6556 | T | C | - | 21.90 |
| E | 1863 | A | G | - | 21.82 | NS4A | 6562 | A | G | Thr63Ala | 22.43 |
| E | 1920 | T | G | - | 17.86 | NS4A | 6564 | A | G | - | 17.43 |
| E | 1994 | C | CGATTG | Val354fs | 2.78 | NS4A | 6585 | A | G | - | 30.22 |
| E | 1995 | G | A | - | 13.89 | NS4A | 6633 | C | T | - | 23.53 |
| E | 2007 | C | T | - | 4.44 | NS4A | 6654 | C | T | - | 21.60 |
| E | 2023 | G | A | Ala363Ser | 12.50 | NS4A | 6681 | G | A | - | 17.04 |
| E | 2024 | C | G | | 12.50 | NS4A | 6711 | G | GT | Leu115fs | 3.08 |
| E | 2223 | T | C | - | 3.29 | NS4B | 6841 | T | C | - | 19.58 |
| E | 2247 | C | T | - | 16.00 | NS4B | 6844 | G | A | Glu7Lys | 2.74 |
| E | 2276 | T | C | Val447Ala | 11.72 | NS4B | 6864 | C | T | - | 12.88 |
| E | 2289 | T | C | - | 10.37 | NS4B | 6871 | G | A | Gly16Arg | 10.98 |
| E | 2307 | T | C | - | 11.68 | NS4B | 6879 | C | T | - | 11.05 |
| E | 2328 | A | T | - | 10.31 | NS4B | 6948 | C | T | - | 10.16 |
| NS1 | 2434 | G | A | Val5Ile | 12.32 | NS4B | 6982 | C | T | Arg53* | 1.47 |
| NS1 | 2451 | C | T | - | 11.29 | NS4B | 7008 | G | A | - | 11.38 |
| NS1 | 2484 | C | T | - | 17.39 | NS4B | 7029 | T | A | - | 8.36 |
| NS1 | 2511 | A | G | - | 10.87 | NS4B | 7038 | G | A | - | 14.96 |
| NS1 | 2589 | G | A | - | 8.13 | NS4B | 7041 | C | T | - | 10.61 |
| NS1 | 2781 | A | G | - | 15.18 | NS4B | 7062 | A | G | - | 10.00 |
| NS1 | 2878 | A | C | Met153Leu | 11.11 | NS4B | 7248 | T | C | - | 10.91 |
| NS1 | 2898 | C | T | - | 11.90 | NS4B | 7314 | G | A | - | 7.00 |
| NS1 | 2911 | A | T | Thr164Ser | 5.63 | NS4B | 7356 | T | C | - | 7.21 |
| NS1 | 2952 | A | G | - | 10.81 | NS4B | 7383 | C | T | - | 10.26 |
| NS1 | 2961 | T | C | - | 16.46 | NS4B | 7428 | T | C | - | 11.49 |
| NS1 | 2976 | G | A | - | 22.50 | NS4B | 7518 | T | C | - | 10.53 |
| NS1 | 2985 | A | T | - | 27.38 | NS5 | 7719 | A | G | - | 28.13 |
| NS1 | 2991 | C | T | - | 16.13 | NS5 | 7758 | G | A | - | 17.14 |
| NS1 | 3075 | C | T | - | 22.73 | NS5 | 7812 | C | T | - | 21.21 |
| NS1 | 3114 | C | A | - | 18.18 | NS5 | 7959 | T | C | - | 6.11 |
| NS1 | 3129 | A | G | - | 8.82 | NS5 | 7999 | C | T | - | 4.15 |
| NS1 | 3234 | C | T | - | 15.38 | NS5 | 8049 | G | A | - | 6.81 |
| NS1 | 3402 | C | T | - | 15.48 | NS5 | 8074 | G | C | Glu169Gln | 2.48 |
| NS1 | 3432 | C | T | - | 15.11 | NS5 | 8083 | T | C | - | 8.00 |
| NS1 | 3438 | G | A | - | 12.59 | NS5 | 8103 | T | C | - | 8.57 |

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|---|---|---|---|---|---|
| NS1 | 3447 | G | A | - | 12.59 |
| NS1 | 3474 | G | A | - | 3.31 |
| NS1 | 3477 | T | C | - | 8.26 |
| NS2A | 3489 | G | A | - | 8.87 |
| NS2A | 3593 | C | CT | Val41fs | 1.98 |
| NS2A | 3720 | C | CA | Val83fs | 1.33 |
| NS2A | 3753 | T | C | - | 11.21 |
| NS2A | 3768 | T | C | - | 12.32 |
| NS2A | 3801 | C | T | - | 5.72 |
| NS2A | 3844 | T | C | - | 17.11 |
| NS2A | 3864 | A | G | - | 10.34 |
| NS2A | 3875 | T | C | Val133Ala | 8.61 |
| NS2A | 3876 | C | T | - | 7.69 |
| NS2A | 3879 | A | C | - | 8.26 |
| NS2A | 3910 | T | C | - | 5.72 |
| NS2A | 3921 | C | T | - | 12.24 |
| NS2A | 4011 | C | T | - | 8.94 |
| NS2A | 4063 | T | C | - | 12.54 |
| NS2A | 4083 | T | C | - | 4.76 |
| NS2B | 4164 | T | C | - | 6.10 |
| NS2B | 4206 | C | T | - | 18.89 |
| NS2B | 4251 | G | A | - | 14.74 |
| NS2B | 4278 | T | C | - | 12.90 |
| NS2B | 4281 | T | C | - | 19.35 |
| NS2B | 4329 | G | A | - | 9.09 |
| NS2B | 4491 | G | A | - | 13.19 |
| NS2B | 4509 | T | G | - | 14.81 |
| NS3 | 4587 | T | C | - | 15.96 |
| NS3 | 4596 | T | C | - | 9.20 |
| NS3 | 4746 | C | T | - | 17.07 |
| NS3 | 4764 | G | A | - | 20.75 |
| NS3 | 4806 | C | T | - | 11.65 |
| NS3 | 4812 | T | C | - | 11.61 |
| NS3 | 4830 | G | A | - | 10.77 |
| NS3 | 4854 | G | A | - | 8.39 |
| NS5 | 8121 | C | T | - | 8.51 |
| NS5 | 8127 | T | C | - | 8.46 |
| NS5 | 8184 | G | A | - | 19.15 |
| NS5 | 8241 | C | T | - | 18.42 |
| NS5 | 8284 | C | T | - | 14.81 |
| NS5 | 8310 | C | T | - | 26.67 |
| NS5 | 8469 | T | C | - | 9.30 |
| NS5 | 8544 | T | C | - | 12.50 |
| NS5 | 8547 | G | A | - | 23.94 |
| NS5 | 8569 | G | A | Val334Ile | 5.88 |
| NS5 | 8574 | C | T | - | 5.48 |
| NS5 | 8745 | G | A | - | 40.00 |
| NS5 | 8855 | G | A | Ser429Asn | 27.45 |
| NS5 | 8967 | T | C | - | 15.79 |
| NS5 | 9066 | T | C | - | 27.91 |
| NS5 | 9079 | T | C | - | 34.48 |
| NS5 | 9112 | T | C | - | 12.90 |
| NS5 | 9369 | C | T | - | 20.00 |
| NS5 | 9402 | C | T | - | 13.64 |
| NS5 | 9501 | C | G | His644Gln | 8.20 |
| NS5 | 9628 | G | A | Val687Ile | 30.43 |
| NS5 | 9825 | C | T | - | 9.09 |
| NS5 | 9840 | T | C | - | 5.59 |
| NS5 | 9849 | A | G | - | 9.64 |
| NS5 | 9942 | C | T | - | 20.97 |
| NS5 | 10059 | A | G | - | 25.74 |
| NS5 | 10083 | T | C | - | 13.51 |
| NS5 | 10152 | A | G | - | 38.10 |
| NS5 | 10155 | C | T | - | 17.78 |
| NS5 | 10202 | A | G | Glu878Gly | 26.47 |
| 3UTR | 10389 | CA | C | - | 3.40 |
| 3UTR | 10389 | CAA | C | - | 26.10 |
| 3UTR | 10409 | C | T | - | 16.84 |
| 3UTR | 10445 | T | TA | - | 6.59 |
| 3UTR | 10613 | C | CAA | - | 9.52 |

| **163** | | | | | |
|---|---|---|---|---|---|
| DF | | | | | |
| Secondary | | | | | |
| 8.64E+04 | | | | | |
| 2 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 102 | C | T | - | 45.71 |
| C | 127 | A | T | Thr11Ser | 9.41 |
| C | 135 | C | T | - | 8.82 |
| C | 336 | C | T | - | 8.47 |
| C | 341 | G | T | Arg82Ile | 3.89 |
| prM/M | 450 | T | C | - | 2.67 |
| prM/M | 457 | A | C | Asn7His | 2.14 |
| prM/M | 606 | C | T | - | 11.83 |
| prM/M | 703 | G | GA | Arg91fs | 5.88 |
| prM/M | 864 | T | C | - | 1.24 |
| prM/M | 912 | A | G | - | 13.37 |
| E | 954 | C | A | - | 3.75 |
| E | 1040 | C | CA | Asn37fs | 7.66 |
| E | 1094 | C | T | Pro53Leu | 5.52 |
| E | 1214 | A | G | Lys93Arg | 6.08 |
| E | 1266 | G | A | - | 2.92 |
| E | 1287 | T | C | - | 2.78 |
| NS3 | 5568 | A | G | - | 2.03 |
| NS3 | 5577 | G | A | - | 2.71 |
| NS3 | 5717 | C | T | Ala399Val | 3.41 |
| NS3 | 5730 | C | T | - | 4.42 |
| NS3 | 5775 | G | A | - | 2.09 |
| NS3 | 5823 | A | G | - | 2.04 |
| NS3 | 5832 | C | T | - | 3.96 |
| NS3 | 5835 | G | A | - | 5.43 |
| NS3 | 5915 | C | CA | Asn467fs | 5.97 |
| NS3 | 6051 | A | G | - | 2.24 |
| NS3 | 6222 | C | T | - | 2.40 |
| NS3 | 6262 | A | G | Ile581Val | 4.51 |
| NS3 | 6340 | C | T | - | 5.94 |
| NS4A | 6824 | CA | C | Asn151fs | 1.26 |
| NS4B | 6844 | G | GA | Thr9fs | 1.13 |
| NS4B | 6951 | C | A | - | 1.26 |
| NS4B | 6982 | C | T | Arg53* | 1.22 |
| NS4B | 7038 | A | G | - | 1.91 |
| NS4B | 7059 | C | T | - | 1.82 |
| NS4B | 7083 | A | G | - | 14.58 |
| NS4B | 7092 | T | C | - | 13.74 |
| NS4B | 7134 | C | T | - | 2.51 |
| NS4B | 7137 | C | T | - | 2.06 |

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|---|---|---|---|---|---|
| E | 1299 | C | CA | Asn124fs | 1.24 |
| E | 1311 | G | A | Met125Ile | 2.05 |
| E | 1689 | T | C | - | 3.72 |
| E | 1710 | T | G | - | 26.09 |
| E | 1834 | T | G | Ser300Ala | 11.27 |
| E | 1860 | T | C | - | 13.01 |
| E | 2256 | C | T | - | 5.84 |
| E | 2318 | T | C | Val461Ala | 7.31 |
| E | 2319 | A | C | - | 2.12 |
| NS1 | 2448 | G | A | - | 2.44 |
| NS1 | 2490 | T | C | - | 2.39 |
| NS1 | 2731 | T | C | - | 3.07 |
| NS1 | 2791 | C | T | Leu124Phe | 1.96 |
| NS1 | 2826 | C | T | - | 11.59 |
| NS1 | 3148 | A | G | Ile243Val | 7.08 |
| NS1 | 3152 | C | CA | Asn246fs | 3.47 |
| NS1 | 3249 | CT | C | Phe277fs | 0.74 |
| NS1 | 3342 | C | T | - | 6.94 |
| NS2A | 3489 | G | A | - | 3.02 |
| NS2A | 3624 | C | T | - | 1.62 |
| NS2A | 3720 | C | CA | Val83fs | 0.42 |
| NS2A | 4006 | G | A | Val177Ile | 2.63 |
| NS2A | 4034 | G | A | Arg186Gln | 1.42 |
| NS2A | 4069 | G | A | Val198Ile | 15.65 |
| NS2A | 4110 | C | T | - | 2.05 |
| NS2B | 4341 | C | T | - | 2.95 |
| NS2B | 4443 | G | T | - | 3.79 |
| NS3 | 4562 | G | A | Gly14Glu | 6.07 |
| NS3 | 4662 | C | T | - | 14.49 |
| NS3 | 4707 | A | G | - | 22.39 |
| NS3 | 4830 | G | A | - | 2.16 |
| NS3 | 4908 | C | T | - | 1.14 |
| NS3 | 4974 | C | T | - | 3.29 |
| NS3 | 5026 | G | GA | Ser171fs | 4.46 |
| NS3 | 5120 | C | CA | Lys201fs | 0.91 |
| NS3 | 5190 | T | C | - | 2.11 |
| NS4B | 7449 | T | C | - | 7.19 |
| NS4B | 7461 | T | C | - | 1.26 |
| NS4B | 7551 | T | C | - | 5.86 |
| NS5 | 7599 | A | G | - | 7.87 |
| NS5 | 7645 | C | A | Gln26Lys | 3.23 |
| NS5 | 7656 | A | G | - | 15.32 |
| NS5 | 7767 | C | T | - | 3.85 |
| NS5 | 7860 | G | A | - | 6.46 |
| NS5 | 7983 | A | G | - | 2.96 |
| NS5 | 7996 | C | T | - | 13.66 |
| NS5 | 7999 | T | C | - | 2.59 |
| NS5 | 8074 | G | C | Glu169Gln | 1.83 |
| NS5 | 8139 | C | T | - | 1.88 |
| NS5 | 8143 | G | GA | Met194fs | 1.72 |
| NS5 | 8211 | C | T | - | 7.20 |
| NS5 | 8298 | T | C | - | 2.36 |
| NS5 | 8331 | A | G | - | 3.76 |
| NS5 | 8602 | G | A | Asp345Asn | 2.37 |
| NS5 | 8604 | C | T | - | 3.98 |
| NS5 | 8619 | G | A | - | 5.91 |
| NS5 | 8891 | T | TA | His442fs | 2.61 |
| NS5 | 8926 | A | AC | Asn453fs | 2.40 |
| NS5 | 9042 | T | C | - | 9.52 |
| NS5 | 9322 | T | C | Ser585Pro | 19.44 |
| NS5 | 9354 | G | A | - | 9.09 |
| NS5 | 9411 | T | C | - | 4.12 |
| NS5 | 9477 | T | C | - | 12.08 |
| NS5 | 9576 | C | T | - | 3.47 |
| NS5 | 9795 | A | G | - | 1.64 |
| NS5 | 9810 | T | C | - | 1.92 |
| NS5 | 9902 | C | G | Ala778Gly | 1.67 |
| NS5 | 10083 | T | C | - | 9.95 |
| NS5 | 10089 | G | A | - | 10.51 |
| NS5 | 10253 | T | A | Met895Lys | 19.61 |
| 3UTR | 10389 | CA | C | - | 10.90 |
| 3UTR | 10389 | C | CA | - | 2.00 |

| **166** | | | | | |
|---|---|---|---|---|---|
| DF | | | | | |
| Primary | | | | | |
| 5.28E+05 | | | | | |
| 1 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 96 | G | A | - | 1.63 |
| C | 135 | C | T | - | 1.92 |
| C | 341 | G | T | Arg82Ile | 0.93 |
| prM/M | 457 | A | C | Asn7His | 0.87 |
| prM/M | 723 | C | T | - | 20.61 |
| prM/M | 734 | T | TGGAACAAGC | Val99Gly100insGluGlnAla | 1.06 |
| prM/M | 737 | G | A | Gly100Glu | 0.69 |
| prM/M | 765 | A | C | - | 0.62 |
| prM/M | 820 | C | T | - | 38.16 |
| prM/M | 834 | C | T | - | 0.50 |
| prM/M | 912 | G | A | - | 27.85 |
| E | 954 | A | C | - | 24.97 |
| E | 1040 | C | CA | Asn37fs | 7.43 |
| E | 1287 | T | C | - | 0.63 |
| E | 1302 | G | A | - | 29.37 |
| E | 1311 | G | A | Met125Ile | 1.23 |
| NS3 | 6105 | A | G | - | 15.92 |
| NS3 | 6155 | G | A | Trp545* | 0.76 |
| NS3 | 6285 | G | A | - | 1.62 |
| NS3 | 6324 | C | T | - | 25.40 |
| NS3 | 6365 | C | T | Ala615Val | 1.32 |
| NS4A | 6418 | A | G | Thr15Ala | 0.75 |
| NS4A | 6606 | C | T | - | 28.78 |
| NS4A | 6652 | G | A | Val93Ile | 27.96 |
| NS4A | 6711 | G | GT | Leu115fs | 6.30 |
| NS4A | 6790 | A | T | Ile139Leu | 1.17 |
| NS4B | 6844 | G | A | Glu7Lys | 1.12 |
| NS4B | 6913 | A | G | Ile30Val | 0.70 |
| NS4B | 6922 | C | T | Arg33Cys | 1.27 |
| NS4B | 6943 | C | T | - | 37.42 |
| NS4B | 6958 | A | G | Thr45Ala | 0.56 |
| NS4B | 6966 | C | T | - | 39.51 |
| NS4B | 6982 | C | T | Arg53* | 0.88 |
| NS4B | 6984 | G | A | - | 37.64 |
| NS4B | 7038 | G | A | - | 35.78 |
| NS4B | 7059 | T | C | - | 33.56 |
| NS4B | 7083 | G | A | - | 33.04 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1479 | T | C | - | 33.48 | NS4B | 7092 | C | T | - | 35.06 |
| E | 1595 | G | T | Trp220Leu | 1.38 | NS4B | 7137 | T | C | - | 35.67 |
| E | 1695 | A | G | - | 0.80 | NS4B | 7158 | T | G | - | 0.70 |
| E | 1701 | C | T | - | 27.60 | NS4B | 7236 | A | T | - | 0.51 |
| E | 1748 | A | T | Gln271Leu | 0.61 | NS4B | 7435 | C | G | Leu204Val | 0.50 |
| E | 1860 | C | T | - | 46.13 | NS4B | 7542 | C | T | - | 37.44 |
| E | 1986 | T | C | - | 33.24 | NS4B | 7551 | T | C | - | 35.53 |
| E | 1992 | T | C | - | 1.09 | NS5 | 7619 | T | G | Leu17Trp | 0.75 |
| E | 1994 | C | CGATTG | Val354fs | 1.77 | NS5 | 7645 | C | A | Gln26Lys | 0.74 |
| E | 2012 | C | T | Thr359Ile | 0.56 | NS5 | 7720 | G | A | Asp51Asn | 0.64 |
| E | 2169 | A | G | - | 19.17 | NS5 | 7748 | C | CA | Leu62fs | 0.53 |
| E | 2210 | T | A | Leu425* | 0.83 | NS5 | 7758 | G | A | - | 13.14 |
| E | 2220 | G | A | - | 41.41 | NS5 | 7763 | T | C | Phe65Ser | 3.12 |
| E | 2223 | T | C | - | 45.28 | NS5 | 7767 | T | C | - | 14.51 |
| E | 2244 | T | C | - | 36.63 | NS5 | 7920 | A | C | - | 0.66 |
| E | 2276 | T | C | Val447Ala | 33.92 | NS5 | 7996 | T | C | - | 22.03 |
| E | 2303 | A | G | Lys456Arg | 1.11 | NS5 | 7999 | C | T | - | 22.01 |
| E | 2308 | C | T | Leu458Phe | 1.31 | NS5 | 8074 | G | C | Glu169Gln | 0.99 |
| E | 2315 | G | T | Gly460Val | 0.92 | NS5 | 8184 | G | C | Leu205Phe | 0.53 |
| E | 2319 | C | A | - | 34.77 | NS5 | 8190 | G | A | - | 0.61 |
| E | 2397 | A | G | - | 31.91 | NS5 | 8298 | C | T | - | 24.53 |
| NS1 | 2448 | A | G | - | 32.03 | NS5 | 8306 | A | C | Lys246Thr | 0.91 |
| NS1 | 2494 | G | A | Val25Met | 29.29 | NS5 | 8448 | T | C | - | 31.11 |
| NS1 | 2620 | A | G | Met67Val | 0.51 | NS5 | 8499 | A | T | Glu310Asp | 0.56 |
| NS1 | 3016 | G | A | Gly199Ser | 0.53 | NS5 | 8544 | T | C | - | 47.84 |
| NS1 | 3152 | C | CA | Asn246fs | 8.73 | NS5 | 8562 | C | T | - | 48.46 |
| NS1 | 3241 | G | GCCT | Glu274delinsAlaTer | 3.96 | NS5 | 8602 | G | A | Asp345Asn | 1.18 |
| NS1 | 3330 | C | T | - | 33.58 | NS5 | 8619 | A | G | - | 44.72 |
| NS2A | 3716 | C | T | Ala80Val | 0.71 | NS5 | 8640 | A | G | - | 44.88 |
| NS2A | 3720 | C | CA | Val83fs | 1.21 | NS5 | 8658 | T | C | - | 38.53 |
| NS2A | 3866 | G | C | Gly130Ala | 1.17 | NS5 | 8760 | A | G | - | 44.52 |
| NS2A | 4034 | A | G | Gln186Arg | 24.59 | NS5 | 8848 | G | C | Glu427Gln | 0.69 |
| NS2A | 4069 | A | G | Ile198Val | 22.71 | NS5 | 8891 | T | TA | His442fs | 1.65 |
| NS2B | 4161 | A | T | - | 0.59 | NS5 | 8899 | G | C | Glu444Gln | 0.64 |
| NS2B | 4188 | T | C | - | 0.64 | NS5 | 8926 | A | AC | Asn453fs | 2.31 |
| NS2B | 4341 | T | C | - | 20.26 | NS5 | 8932 | A | T | Met455Leu | 0.86 |
| NS2B | 4482 | G | A | - | 31.37 | NS5 | 9208 | T | C | - | 16.99 |
| NS3 | 4707 | G | A | - | 41.46 | NS5 | 9226 | G | A | Val553Ile | 19.78 |
| NS3 | 4863 | A | G | - | 0.50 | NS5 | 9303 | A | G | - | 38.61 |
| NS3 | 4974 | T | C | - | 27.02 | NS5 | 9322 | C | T | Pro585Ser | 35.31 |
| NS3 | 5026 | G | A | Glu169Lys | 0.56 | NS5 | 9399 | C | T | - | 43.45 |
| NS3 | 5026 | G | GA | Ser171fs | 6.40 | NS5 | 9424 | C | T | - | 42.04 |
| NS3 | 5033 | G | A | Ser171Asn | 1.93 | NS5 | 9468 | G | C | Gln633His | 0.51 |
| NS3 | 5037 | T | C | - | 0.72 | NS5 | 9477 | T | C | - | 40.12 |
| NS3 | 5304 | T | C | - | 44.59 | NS5 | 9576 | T | C | - | 45.27 |
| NS3 | 5304 | T | G | Cys261Trp | 1.41 | NS5 | 9660 | T | A | - | 38.61 |
| NS3 | 5394 | G | A | - | 43.70 | NS5 | 9705 | T | TC | Phe713fs | 0.82 |
| NS3 | 5433 | A | T | - | 1.42 | NS5 | 9725 | T | A | Ile719Lys | 39.11 |
| NS3 | 5436 | G | A | - | 1.79 | NS5 | 9768 | G | A | - | 39.04 |
| NS3 | 5531 | G | T | Arg337Ile | 1.17 | NS5 | 9789 | C | T | - | 38.86 |
| NS3 | 5543 | A | C | Glu341Ala | 1.14 | NS5 | 9795 | G | A | - | 38.53 |
| NS3 | 5642 | G | T | Cys374Phe | 0.62 | NS5 | 9877 | C | T | Arg770Cys | 0.70 |
| NS3 | 5730 | T | C | - | 37.54 | NS5 | 9902 | C | G | Ala778Gly | 2.48 |
| NS3 | 5835 | A | G | - | 31.47 | NS5 | 9942 | C | T | - | 38.31 |
| NS3 | 5839 | C | T | Arg440Trp | 1.58 | 3UTR | 10389 | T | C | - | 0.97 |
| NS3 | 5915 | C | CA | Asn467fs | 7.09 | 3UTR | 10502 | C | A | - | 0.76 |
| NS3 | 5915 | C | CAA | Asn467fs | 0.90 | 3UTR | 10608 | C | CA | - | 0.52 |
| NS3 | 6099 | G | A | - | 16.60 | 3UTR | 10631 | C | T | - | 0.55 |

| **167** | | | | | |
|---|---|---|---|---|---|
| DF | | | | | |
| Primary | | | | | |
| 4.79E+05 | | | | | |
| 3 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 201 | C | T | - | 11.82 |
| C | 315 | A | G | - | 23.53 |
| C | 327 | T | C | - | 23.58 |
| C | 336 | C | T | - | 21.77 |
| C | 348 | T | C | - | 17.71 |
| C | 384 | T | C | - | 9.34 |
| C | 387 | A | G | - | 9.78 |
| prM/M | 445 | C | T | - | 8.01 |
| prM/M | 450 | T | C | - | 8.75 |
| prM/M | 508 | T | C | - | 14.17 |
| prM/M | 567 | A | G | - | 9.56 |
| prM/M | 651 | A | G | - | 3.45 |
| prM/M | 703 | G | GA | Arg91fs | 7.55 |
| prM/M | 792 | A | G | - | 4.15 |
| prM/M | 798 | C | T | - | 4.02 |
| prM/M | 834 | C | T | - | 6.13 |
| prM/M | 837 | C | T | - | 6.00 |
| prM/M | 852 | A | G | - | 7.81 |
| prM/M | 856 | T | C | - | 7.46 |
| prM/M | 864 | T | C | - | 5.99 |
| prM/M | 873 | C | A | - | 6.42 |
| prM/M | 894 | A | C | - | 10.34 |
| prM/M | 912 | A | G | - | 17.09 |
| prM/M | 921 | C | T | - | 12.36 |
| E | 954 | C | A | - | 18.13 |
| E | 1005 | A | C | - | 11.29 |
| E | 1040 | C | CA | Asn37fs | 4.33 |
| E | 1102 | T | C | - | 13.74 |
| E | 1293 | C | T | - | 3.85 |
| E | 1353 | C | T | - | 3.88 |
| E | 1413 | C | A | - | 3.29 |
| E | 1590 | A | G | - | 4.39 |
| E | 1595 | G | T | Trp220Leu | 1.05 |
| E | 1641 | A | G | - | 8.25 |
| E | 1842 | T | C | - | 6.25 |
| E | 1860 | T | C | - | 12.96 |
| E | 1863 | A | G | - | 9.43 |
| E | 1995 | G | A | - | 5.84 |
| E | 2023 | G | A | Ala363Ser | 6.87 |
| E | 2024 | C | G | | 6.81 |
| E | 2049 | T | C | - | 2.04 |
| E | 2064 | C | T | - | 1.45 |
| E | 2223 | C | T | - | 10.10 |
| E | 2247 | C | T | - | 9.88 |
| E | 2289 | T | C | - | 7.05 |
| E | 2307 | T | C | - | 10.35 |
| E | 2319 | A | C | - | 9.19 |
| E | 2328 | A | T | - | 6.21 |
| NS1 | 2434 | G | A | Val5Ile | 6.21 |
| NS1 | 2448 | G | A | - | 10.11 |
| NS1 | 2451 | C | T | - | 6.56 |
| NS1 | 2484 | C | T | - | 5.98 |
| NS1 | 2511 | A | G | - | 4.53 |
| NS1 | 2589 | G | A | - | 4.72 |
| NS3 | 5004 | T | C | - | 11.81 |
| NS3 | 5026 | G | GA | Ser171fs | 5.03 |
| NS3 | 5070 | T | C | - | 10.43 |
| NS3 | 5190 | T | C | - | 12.60 |
| NS3 | 5235 | T | C | - | 12.54 |
| NS3 | 5277 | T | C | - | 14.22 |
| NS3 | 5283 | G | A | - | 13.31 |
| NS3 | 5304 | C | T | - | 9.38 |
| NS3 | 5531 | G | T | Arg337Ile | 2.60 |
| NS3 | 5613 | C | T | - | 6.77 |
| NS3 | 5730 | C | T | - | 2.55 |
| NS3 | 5769 | T | C | - | 11.82 |
| NS3 | 5796 | T | C | - | 7.00 |
| NS3 | 5802 | T | C | - | 6.32 |
| NS3 | 5835 | G | A | - | 8.74 |
| NS3 | 5844 | G | A | - | 2.12 |
| NS3 | 5847 | T | C | - | 5.03 |
| NS3 | 6009 | C | T | - | 9.42 |
| NS3 | 6069 | T | G | - | 12.76 |
| NS3 | 6094 | C | T | - | 8.42 |
| NS3 | 6099 | G | A | - | 8.45 |
| NS3 | 6155 | G | A | Trp545* | 1.31 |
| NS3 | 6220 | G | A | Val567Ile | 4.21 |
| NS3 | 6222 | C | T | - | 4.29 |
| NS3 | 6300 | T | C | - | 26.90 |
| NS3 | 6330 | C | T | - | 16.60 |
| NS4A | 6378 | C | T | - | 12.79 |
| NS4A | 6379 | C | T | - | 13.20 |
| NS4A | 6435 | G | A | - | 1.29 |
| NS4A | 6462 | C | T | - | 9.52 |
| NS4A | 6471 | T | C | - | 7.72 |
| NS4A | 6491 | G | A | Arg39Lys | 11.73 |
| NS4A | 6496 | C | T | His41Tyr | 11.98 |
| NS4A | 6500 | A | C | Asn42Thr | 9.88 |
| NS4A | 6528 | C | T | - | 11.97 |
| NS4A | 6547 | T | C | - | 11.04 |
| NS4A | 6556 | T | C | - | 11.26 |
| NS4A | 6562 | A | G | Thr63Ala | 10.36 |
| NS4A | 6564 | A | G | - | 9.49 |
| NS4A | 6585 | A | G | - | 14.99 |
| NS4A | 6633 | C | T | - | 11.97 |
| NS4A | 6654 | C | T | - | 10.70 |
| NS4A | 6681 | G | A | - | 12.86 |
| NS4A | 6711 | G | GT | Leu115fs | 3.40 |
| NS4B | 6841 | T | C | - | 15.02 |
| NS4B | 6844 | G | A | Glu7Lys | 2.62 |
| NS4B | 6864 | C | T | - | 15.00 |
| NS4B | 6871 | G | A | Gly16Arg | 14.85 |
| NS4B | 6879 | C | T | - | 15.19 |
| NS4B | 6948 | C | T | - | 5.49 |
| NS4B | 6982 | C | T | Arg53* | 1.25 |
| NS4B | 7008 | G | A | - | 3.42 |
| NS4B | 7029 | T | A | - | 4.03 |
| NS4B | 7038 | A | G | - | 2.63 |
| NS4B | 7041 | C | T | - | 4.89 |
| NS4B | 7059 | C | T | - | 7.11 |
| NS4B | 7062 | A | G | - | 4.39 |
| NS4B | 7083 | A | G | - | 7.33 |
| NS4B | 7137 | C | T | - | 7.39 |
| NS4B | 7165 | C | T | - | 2.96 |

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|---|---|---|---|---|---|
| NS1 | 2677 | T | C | - | 20.80 |
| NS1 | 2781 | A | G | - | 5.88 |
| NS1 | 2878 | A | C | Met153Leu | 3.74 |
| NS1 | 2898 | C | T | - | 3.78 |
| NS1 | 2952 | A | G | - | 6.01 |
| NS1 | 2961 | T | C | - | 6.41 |
| NS1 | 2976 | G | A | - | 10.53 |
| NS1 | 2991 | C | T | - | 9.30 |
| NS1 | 3075 | C | T | - | 10.91 |
| NS1 | 3114 | C | A | - | 4.22 |
| NS1 | 3152 | C | CA | Asn246fs | 7.69 |
| NS1 | 3234 | C | T | - | 9.13 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 1.72 |
| NS1 | 3330 | T | C | - | 12.31 |
| NS1 | 3398 | A | G | Glu326Gly | 2.29 |
| NS1 | 3402 | C | T | - | 7.66 |
| NS1 | 3432 | C | T | - | 9.59 |
| NS1 | 3438 | G | A | - | 8.56 |
| NS1 | 3447 | G | A | - | 8.68 |
| NS1 | 3474 | G | A | - | 7.83 |
| NS2A | 3597 | T | C | - | 2.64 |
| NS2A | 3624 | C | T | - | 5.25 |
| NS2A | 3629 | A | G | Lys51Arg | 5.39 |
| NS2A | 3716 | C | T | Ala80Val | 0.84 |
| NS2A | 3720 | C | CA | Val83fs | 1.77 |
| NS2A | 3753 | T | C | - | 11.72 |
| NS2A | 3768 | T | C | - | 12.05 |
| NS2A | 3801 | C | T | - | 2.53 |
| NS2A | 3864 | A | G | - | 12.11 |
| NS2A | 3875 | T | C | Val133Ala | 11.98 |
| NS2A | 3876 | C | T | - | 10.71 |
| NS2A | 3879 | A | C | - | 11.64 |
| NS2A | 3910 | T | C | - | 2.71 |
| NS2A | 3921 | C | T | - | 10.31 |
| NS2A | 3928 | G | C | Ala151Pro | 1.67 |
| NS2A | 4011 | C | T | - | 7.05 |
| NS2A | 4034 | G | A | Arg186Gln | 10.32 |
| NS2A | 4063 | T | C | - | 7.58 |
| NS2A | 4069 | G | A | Val198Ile | 12.38 |
| NS2A | 4083 | T | C | - | 7.23 |
| NS2A | 4110 | C | T | - | 13.07 |
| NS2B | 4164 | T | C | - | 5.47 |
| NS2B | 4206 | C | T | - | 8.37 |
| NS2B | 4249 | G | A | Val40Ile | 2.92 |
| NS2B | 4257 | C | T | - | 1.95 |
| NS2B | 4278 | T | C | - | 6.73 |
| NS2B | 4329 | G | A | - | 5.45 |
| NS2B | 4338 | G | A | - | 3.91 |
| NS2B | 4344 | T | C | - | 4.21 |
| NS2B | 4350 | C | T | - | 4.81 |
| NS2B | 4414 | T | C | - | 9.72 |
| NS2B | 4443 | G | T | - | 6.29 |
| NS2B | 4482 | A | G | - | 6.83 |
| NS2B | 4491 | G | A | - | 3.08 |
| NS2B | 4509 | T | G | - | 3.50 |
| NS3 | 4596 | T | C | - | 9.78 |
| NS3 | 4707 | A | G | - | 5.33 |
| NS3 | 4746 | C | T | - | 6.48 |
| NS3 | 4806 | C | T | - | 6.95 |
| NS3 | 4812 | T | C | - | 5.37 |
| NS4B | 7248 | T | C | - | 4.48 |
| NS4B | 7314 | G | A | - | 9.00 |
| NS4B | 7356 | T | C | - | 6.06 |
| NS4B | 7383 | C | T | - | 6.41 |
| NS4B | 7428 | T | C | - | 3.54 |
| NS4B | 7518 | T | C | - | 6.82 |
| NS4B | 7551 | T | C | - | 5.16 |
| NS5 | 7656 | A | G | - | 18.82 |
| NS5 | 7776 | T | C | - | 9.09 |
| NS5 | 7812 | C | T | - | 4.30 |
| NS5 | 7911 | T | C | - | 5.08 |
| NS5 | 7959 | T | C | - | 5.66 |
| NS5 | 7996 | C | T | - | 6.23 |
| NS5 | 7998 | G | T | Leu143Phe | 1.03 |
| NS5 | 7999 | T | C | - | 2.74 |
| NS5 | 8049 | G | A | - | 6.23 |
| NS5 | 8083 | T | C | - | 8.83 |
| NS5 | 8103 | T | C | - | 7.17 |
| NS5 | 8121 | C | T | - | 5.07 |
| NS5 | 8127 | T | C | - | 5.21 |
| NS5 | 8184 | G | A | - | 7.38 |
| NS5 | 8241 | C | T | - | 7.30 |
| NS5 | 8244 | T | C | - | 8.88 |
| NS5 | 8284 | C | T | - | 7.47 |
| NS5 | 8298 | T | C | - | 9.81 |
| NS5 | 8310 | C | T | - | 9.71 |
| NS5 | 8380 | G | A | Val271Ile | 13.64 |
| NS5 | 8469 | T | C | - | 3.73 |
| NS5 | 8544 | T | C | - | 7.01 |
| NS5 | 8569 | G | A | Val334Ile | 1.99 |
| NS5 | 8574 | C | T | - | 7.26 |
| NS5 | 8602 | G | A | Asp345Asn | 1.98 |
| NS5 | 8619 | G | A | - | 9.25 |
| NS5 | 8628 | T | C | - | 8.54 |
| NS5 | 8745 | G | A | - | 13.64 |
| NS5 | 8787 | T | C | - | 33.33 |
| NS5 | 8803 | A | G | Ile412Val | 17.24 |
| NS5 | 8926 | A | AC | Asn453fs | 3.52 |
| NS5 | 8967 | T | C | - | 8.63 |
| NS5 | 9006 | T | C | - | 2.22 |
| NS5 | 9066 | T | C | - | 13.82 |
| NS5 | 9079 | T | C | - | 13.87 |
| NS5 | 9112 | T | C | - | 6.52 |
| NS5 | 9396 | T | A | - | 5.48 |
| NS5 | 9399 | C | T | - | 5.48 |
| NS5 | 9402 | C | T | - | 7.89 |
| NS5 | 9424 | C | T | - | 16.67 |
| NS5 | 9477 | T | C | - | 6.80 |
| NS5 | 9501 | C | G | His644Gln | 3.24 |
| NS5 | 9576 | C | T | - | 11.95 |
| NS5 | 9628 | G | A | Val687Ile | 20.43 |
| NS5 | 9795 | A | G | - | 8.72 |
| NS5 | 9825 | C | T | - | 6.31 |
| NS5 | 9840 | T | C | - | 5.40 |
| NS5 | 9849 | A | G | - | 4.34 |
| NS5 | 9902 | C | G | Ala778Gly | 1.08 |
| NS5 | 9942 | T | C | - | 3.34 |
| NS5 | 10083 | T | C | - | 4.38 |
| NS5 | 10089 | GA | G | Arg842fs | 0.75 |
| NS5 | 10155 | C | T | - | 3.74 |

| | | | | | |
|---|---|---|---|---|---|
| NS3 | 4830 | G | A | - | 6.34 |
| NS3 | 4854 | G | A | - | 8.45 |
| NS3 | 4875 | C | T | - | 9.68 |
| NS3 | 4905 | A | G | - | 14.79 |
| NS3 | 4944 | T | C | - | 15.87 |
| NS3 | 4959 | C | T | - | 16.29 |
| NS5 | 10202 | A | G | Glu878Gly | 2.89 |
| 3UTR | 10389 | CA | C | - | 21.20 |
| 3UTR | 10389 | C | CA | - | 2.90 |
| 3UTR | 10408 | C | T | - | 10.70 |
| 3UTR | 10444 | T | TA | - | 3.88 |
| 3UTR | 10612 | C | CAAA | - | 8.51 |

| **168** | | | | | |
|---|---|---|---|---|---|
| DF | | | | | |
| Secondary | | | | | |
| 2.78E+05 | | | | | |
| 2 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 70 | G | GT | - | 2.60 |
| 5UTR | 96 | G | A | - | 1.16 |
| C | 135 | C | T | - | 1.87 |
| C | 148 | C | G | Arg18Gly | 0.76 |
| C | 201 | C | T | - | 1.06 |
| C | 219 | T | C | - | 2.60 |
| C | 341 | G | T | Arg82Ile | 3.31 |
| C | 387 | A | G | - | 0.71 |
| C | 411 | T | C | - | 0.61 |
| C | 431 | C | T | Ala112Val | 0.65 |
| prM/M | 445 | C | T | - | 0.75 |
| prM/M | 457 | A | C | Asn7His | 0.56 |
| prM/M | 508 | T | C | - | 0.78 |
| prM/M | 523 | G | A | Asp29Asn | 0.72 |
| prM/M | 525 | T | C | - | 0.79 |
| prM/M | 531 | T | C | - | 0.89 |
| prM/M | 567 | A | G | - | 1.23 |
| prM/M | 651 | A | G | - | 1.08 |
| prM/M | 703 | G | GA | Arg91fs | 3.74 |
| prM/M | 792 | A | G | - | 0.86 |
| prM/M | 798 | C | T | - | 0.89 |
| prM/M | 820 | T | C | - | 0.63 |
| prM/M | 834 | C | T | - | 1.18 |
| prM/M | 837 | C | T | - | 0.80 |
| prM/M | 852 | A | G | - | 0.97 |
| prM/M | 855 | T | C | - | 1.98 |
| prM/M | 856 | T | C | - | 0.91 |
| prM/M | 864 | T | C | - | 0.97 |
| prM/M | 873 | C | A | - | 1.03 |
| prM/M | 891 | G | C | Arg151Ser | 0.52 |
| prM/M | 894 | A | C | - | 1.29 |
| E | 1005 | A | C | - | 0.65 |
| E | 1023 | T | C | - | 1.10 |
| E | 1040 | C | CA | Asn37fs | 7.67 |
| E | 1102 | T | C | - | 1.52 |
| E | 1313 | A | C | Glu126Ala | 2.36 |
| E | 1352 | T | G | Ile139Ser | 0.65 |
| E | 1368 | T | C | - | 1.26 |
| E | 1473 | T | C | - | 0.75 |
| E | 1545 | C | T | - | 0.58 |
| E | 1595 | G | T | Trp220Leu | 1.95 |
| E | 1599 | A | G | - | 0.64 |
| E | 1626 | T | C | - | 0.75 |
| E | 1641 | A | G | - | 0.69 |
| E | 1695 | A | G | - | 1.42 |
| NS3 | 5264 | G | A | Arg248Lys | 0.52 |
| NS3 | 5304 | T | G | Cys261Trp | 1.86 |
| NS3 | 5309 | C | A | Ala263Asp | 1.13 |
| NS3 | 5433 | A | T | - | 1.66 |
| NS3 | 5436 | G | A | - | 1.71 |
| NS3 | 5531 | G | T | Arg337Ile | 1.32 |
| NS3 | 5543 | A | C | Glu341Ala | 0.69 |
| NS3 | 5575 | A | T | Thr352Ser | 0.85 |
| NS3 | 5648 | G | GA | Lys377fs | 3.02 |
| NS3 | 5717 | C | T | Ala399Val | 1.18 |
| NS3 | 5722 | G | C | Asp401His | 0.48 |
| NS3 | 5730 | T | C | - | 0.64 |
| NS3 | 5754 | A | T | - | 0.77 |
| NS3 | 5775 | A | G | - | 0.58 |
| NS3 | 5793 | T | C | - | 0.80 |
| NS3 | 5823 | G | A | - | 1.09 |
| NS3 | 5839 | C | T | Arg440Trp | 1.44 |
| NS3 | 5847 | T | C | - | 0.75 |
| NS3 | 5859 | T | C | - | 0.81 |
| NS3 | 5915 | C | CA | Asn467fs | 9.70 |
| NS3 | 6009 | C | T | - | 1.42 |
| NS3 | 6033 | C | T | - | 1.66 |
| NS3 | 6061 | G | GA | Val516fs | 0.74 |
| NS3 | 6069 | T | G | - | 4.00 |
| NS3 | 6087 | A | G | - | 2.36 |
| NS3 | 6093 | T | C | - | 3.02 |
| NS3 | 6094 | C | T | - | 7.86 |
| NS3 | 6096 | G | A | - | 3.00 |
| NS3 | 6099 | G | A | - | 8.98 |
| NS3 | 6105 | G | A | - | 11.17 |
| NS3 | 6108 | A | G | - | 4.52 |
| NS3 | 6141 | G | A | - | 3.16 |
| NS3 | 6157 | T | C | - | 3.06 |
| NS3 | 6159 | G | A | - | 2.29 |
| NS3 | 6220 | G | A | Val567Ile | 0.89 |
| NS3 | 6222 | C | T | - | 0.90 |
| NS3 | 6285 | G | A | - | 1.06 |
| NS3 | 6285 | G | GA | Lys590fs | 5.56 |
| NS3 | 6300 | T | C | - | 0.94 |
| NS3 | 6365 | C | T | Ala615Val | 1.19 |
| NS4A | 6462 | C | T | - | 0.93 |
| NS4A | 6466 | T | C | - | 1.74 |
| NS4A | 6491 | G | A | Arg39Lys | 1.25 |
| NS4A | 6496 | C | T | His41Tyr | 1.30 |
| NS4A | 6500 | A | C | Asn42Thr | 1.16 |
| NS4A | 6512 | G | A | Ser46Asn | 1.45 |
| NS4A | 6528 | C | T | - | 1.40 |
| NS4A | 6547 | T | C | - | 1.32 |
| NS4A | 6556 | T | C | - | 1.33 |
| NS4A | 6562 | A | G | Thr63Ala | 1.17 |
| NS4A | 6573 | A | G | - | 0.76 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1920 | T | G | - | 1.08 | NS4A | 6633 | C | T | - | 1.02 |
| E | 1950 | A | G | - | 3.57 | NS4A | 6654 | C | T | - | 1.17 |
| E | 1972 | C | T | His346Tyr | 0.87 | NS4A | 6655 | C | T | Leu94Phe | 1.07 |
| E | 1986 | C | T | - | 0.66 | NS4A | 6681 | G | A | - | 1.07 |
| E | 1994 | C | CGATTG | Val354fs | 2.22 | NS4A | 6711 | G | GT | Leu115fs | 7.49 |
| E | 2012 | C | T | Thr359Ile | 0.94 | NS4A | 6790 | A | T | Ile139Leu | 1.41 |
| E | 2023 | G | A | Ala363Ser | 0.53 | NS4B | 6841 | T | C | - | 1.37 |
| E | 2024 | C | G | | 0.53 | NS4B | 6844 | G | GA | Thr9fs | 3.03 |
| E | 2067 | T | C | - | 0.73 | NS4B | 6844 | G | A | Glu7Lys | 1.28 |
| E | 2194 | T | A | Trp420Arg | 0.75 | NS4B | 6879 | C | T | - | 1.26 |
| E | 2210 | T | A | Leu425* | 0.95 | NS4B | 6922 | C | T | Arg33Cys | 1.06 |
| E | 2247 | C | T | - | 0.69 | NS4B | 6948 | C | T | - | 1.11 |
| E | 2269 | G | A | Gly445Arg | 0.61 | NS4B | 6957 | T | C | - | 1.22 |
| E | 2276 | T | C | Val447Ala | 0.98 | NS4B | 6958 | A | G | Thr45Ala | 0.65 |
| E | 2303 | A | G | Lys456Arg | 1.15 | NS4B | 6966 | T | C | - | 0.69 |
| E | 2307 | T | C | - | 0.88 | NS4B | 6982 | C | T | Arg53* | 1.58 |
| E | 2308 | C | T | Leu458Phe | 1.31 | NS4B | 6984 | A | G | - | 0.56 |
| E | 2315 | G | T | Gly460Val | 1.56 | NS4B | 7008 | G | A | - | 0.87 |
| NS1 | 2451 | C | T | - | 0.80 | NS4B | 7018 | C | T | - | 0.94 |
| NS1 | 2484 | C | T | - | 0.81 | NS4B | 7038 | G | A | - | 0.85 |
| NS1 | 2511 | A | G | - | 0.67 | NS4B | 7041 | C | T | - | 0.84 |
| NS1 | 2961 | T | C | - | 1.02 | NS4B | 7062 | A | G | - | 1.05 |
| NS1 | 2976 | G | A | - | 0.98 | NS4B | 7152 | A | T | - | 0.73 |
| NS1 | 2985 | A | T | - | 1.49 | NS4B | 7158 | T | G | - | 1.15 |
| NS1 | 2989 | G | A | Asp190Asn | 0.82 | NS4B | 7165 | C | T | - | 0.77 |
| NS1 | 3009 | C | T | - | 0.74 | NS4B | 7186 | A | G | ile121Val | 0.88 |
| NS1 | 3075 | C | T | - | 0.81 | NS4B | 7248 | T | C | - | 0.96 |
| NS1 | 3081 | T | A | - | 0.78 | NS4B | 7410 | C | T | - | 0.64 |
| NS1 | 3152 | C | CA | Asn246fs | 8.47 | NS4B | 7471 | A | G | Thr216Ala | 0.73 |
| NS1 | 3234 | C | T | - | 0.87 | NS5 | 7640 | A | C | Glu24Ala | 0.80 |
| NS1 | 3241 | G | GCCT | Glu27AlaTer | 4.00 | NS5 | 7645 | C | A | Gln26Lys | 1.19 |
| NS1 | 3398 | A | G | Glu326Gly | 1.01 | NS5 | 7719 | A | G | - | 0.96 |
| NS1 | 3402 | C | T | - | 0.59 | NS5 | 7748 | C | CA | Leu62fs | 0.98 |
| NS2A | 3503 | C | A | Ser9* | 0.57 | NS5 | 7767 | C | T | - | 1.67 |
| NS2A | 3624 | C | T | - | 0.74 | NS5 | 7812 | C | T | - | 1.03 |
| NS2A | 3629 | A | G | Lys51Arg | 0.68 | NS5 | 7959 | T | C | - | 0.89 |
| NS2A | 3663 | T | C | - | 1.03 | NS5 | 7999 | C | T | - | 0.90 |
| NS2A | 3716 | C | T | Ala80Val | 0.74 | NS5 | 8049 | G | A | - | 0.91 |
| NS2A | 3768 | T | C | - | 0.61 | NS5 | 8074 | G | C | Glu169Gln | 1.77 |
| NS2A | 3844 | T | C | - | 1.63 | NS5 | 8121 | C | T | - | 0.86 |
| NS2A | 3864 | A | G | - | 1.01 | NS5 | 8184 | G | C | Leu205Phe | 0.91 |
| NS2A | 3866 | G | C | Gly130Ala | 2.13 | NS5 | 8184 | G | A | - | 0.69 |
| NS2A | 3875 | T | C | Val133Ala | 1.06 | NS5 | 8547 | G | A | - | 0.84 |
| NS2A | 3879 | A | C | - | 1.01 | NS5 | 8562 | T | C | - | 0.60 |
| NS2A | 3921 | C | T | - | 1.50 | NS5 | 8602 | G | A | Asp345Asn | 1.68 |
| NS2A | 3928 | G | C | Ala151Pro | 1.57 | NS5 | 8796 | G | A | - | 0.67 |
| NS2A | 3935 | C | T | Ser153Leu | 0.50 | NS5 | 8803 | A | G | Ile412Val | 0.98 |
| NS2A | 3998 | C | T | Ala174Val | 0.50 | NS5 | 8891 | T | TA | His442fs | 2.16 |
| NS2A | 4011 | C | T | - | 1.10 | NS5 | 8919 | TG | T | Val451fs | 0.69 |
| NS2A | 4083 | T | C | - | 0.93 | NS5 | 8926 | A | AC | Asn453fs | 3.48 |
| NS2A | 4089 | A | T | - | 0.63 | NS5 | 8932 | A | T | Met455Leu | 1.10 |
| NS2B | 4164 | T | C | - | 1.55 | NS5 | 9006 | T | C | - | 0.54 |
| NS2B | 4206 | C | T | - | 1.87 | NS5 | 9705 | T | TC | Phe713fs | 1.14 |
| NS2B | 4251 | G | A | - | 0.89 | NS5 | 9726 | AG | A | Asp720fs | 0.78 |
| NS2B | 4281 | C | T | - | 1.41 | NS5 | 9825 | C | T | - | 1.30 |
| NS2B | 4350 | C | T | - | 0.89 | NS5 | 9888 | A | G | - | 1.07 |
| NS3 | 4830 | G | A | - | 0.75 | NS5 | 9902 | C | G | Ala778Gly | 2.31 |
| NS3 | 4837 | C | T | Pro106Ser | 0.71 | NS5 | 9942 | C | T | - | 1.11 |
| NS3 | 4905 | A | G | - | 0.68 | NS5 | 10059 | A | G | - | 1.32 |
| NS3 | 4944 | T | C | - | 1.04 | NS5 | 10075 | G | A | Val836Ile | 0.60 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS3 | 4959 | C | T | - | 0.80 | NS5 | 10083 | T | C | - | 0.53 |
| NS3 | 5004 | T | C | - | 0.78 | NS5 | 10148 | C | CA | Asn862fs | 3.20 |
| NS3 | 5026 | G | A | Glu169Lys | 0.67 | NS5 | 10249 | GA | G | Glu895fs | 0.52 |
| NS3 | 5026 | G | GA | Ser171fs | 7.94 | 3UTR | 10389 | CAA | C | - | 11.80 |
| NS3 | 5070 | T | C | - | 0.81 | 3UTR | 10409 | C | T | - | 1.78 |
| NS3 | 5082 | A | G | - | 0.80 | 3UTR | 10601 | A | AC | - | 1.09 |
| NS3 | 5136 | A | T | - | 0.92 | 3UTR | 10613 | C | CA | - | 4.29 |

| **169** | | | | | | NS3 | 5433 | A | T | - | 1.51 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | | | | | | NS3 | 5436 | G | A | - | 1.88 |
| Primary | | | | | | NS3 | 5447 | C | T | Ala309Val | 0.66 |
| 2.31E+05 | | | | | | NS3 | 5531 | G | T | Arg337Ile | 1.24 |
| 2 | | | | | | NS3 | 5543 | A | C | Glu341Ala | 1.54 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5839 | C | T | Arg440Trp | 2.65 |
| 5UTR | 70 | G | GT | - | 3.46 | NS3 | 5915 | C | CA | Asn467fs | 7.89 |
| C | 135 | C | T | - | 1.05 | NS3 | 6155 | G | A | Trp545* | 0.68 |
| C | 341 | G | T | Arg82Ile | 3.15 | NS3 | 6285 | G | A | - | 1.44 |
| prM/M | 457 | A | C | Asn7His | 1.32 | NS3 | 6285 | G | GA | Lys590fs | 3.20 |
| prM/M | 636 | T | G | Cys66Trp | 0.74 | NS3 | 6365 | C | T | Ala615Val | 0.97 |
| prM/M | 703 | G | GA | Arg91fs | 3.53 | NS4A | 6418 | A | G | Thr15Ala | 0.78 |
| prM/M | 802 | A | G | Arg122Gly | 0.54 | NS4A | 6655 | C | T | Leu94Phe | 0.72 |
| prM/M | 811 | A | G | Thr125Ala | 0.67 | NS4A | 6790 | A | T | Ile139Leu | 1.01 |
| prM/M | 834 | C | T | - | 0.82 | NS4B | 6844 | G | A | Glu7Lys | 1.17 |
| E | 1040 | C | CA | Asn37fs | 6.05 | NS4B | 6913 | A | G | Ile30Val | 0.84 |
| E | 1287 | T | C | - | 0.61 | NS4B | 6922 | C | T | Arg33Cys | 1.43 |
| E | 1311 | G | A | Met125Ile | 1.45 | NS4B | 6958 | A | G | Thr45Ala | 0.56 |
| E | 1595 | G | T | Trp220Leu | 1.38 | NS4B | 6982 | C | T | Arg53* | 1.61 |
| E | 1695 | A | G | - | 1.43 | NS4B | 7049 | T | C | Leu75Ser | 0.95 |
| E | 1748 | A | T | Gln271Leu | 0.60 | NS4B | 7471 | A | G | Thr216Ala | 0.63 |
| E | 1994 | C | CGATTG | Val354fs | 1.96 | NS5 | 7619 | T | G | Leu17Trp | 0.60 |
| E | 2012 | C | T | Thr359Ile | 0.63 | NS5 | 7645 | C | A | Gln26Lys | 1.82 |
| E | 2108 | G | GA | Trp391fs | 0.53 | NS5 | 7748 | C | CA | Leu62fs | 0.54 |
| E | 2113 | A | C | Lys393Gln | 0.83 | NS5 | 7920 | A | C | - | 0.66 |
| E | 2210 | T | A | Leu425* | 1.09 | NS5 | 8074 | G | C | Glu169Gln | 1.39 |
| E | 2303 | A | G | Lys456Arg | 1.21 | NS5 | 8258 | C | A | Ser230* | 0.61 |
| E | 2308 | C | T | Leu458Phe | 1.44 | NS5 | 8499 | A | T | Glu310Asp | 1.41 |
| E | 2411 | T | C | Met492Thr | 0.58 | NS5 | 8602 | G | A | Asp345Asn | 1.69 |
| NS1 | 2472 | C | T | - | 0.83 | NS5 | 8712 | T | C | - | 3.21 |
| NS1 | 2665 | A | T | Asn82Tyr | 0.51 | NS5 | 8718 | A | G | - | 5.11 |
| NS1 | 2756 | A | G | Lys112Arg | 0.58 | NS5 | 8727 | A | G | - | 4.17 |
| NS1 | 3093 | C | T | - | 0.75 | NS5 | 8731 | G | A | Glu388Lys | 3.95 |
| NS1 | 3152 | C | CA | Asn246fs | 7.59 | NS5 | 8891 | T | TA | His442fs | 1.22 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 3.40 | NS5 | 8899 | G | C | Glu444Gln | 1.03 |
| NS1 | 3398 | A | G | Glu326Gly | 0.84 | NS5 | 8926 | A | AC | Asn453fs | 4.53 |
| NS2A | 3716 | C | T | Ala80Val | 0.73 | NS5 | 8932 | A | T | Met455Leu | 1.51 |
| NS2A | 3866 | G | C | Gly130Ala | 1.31 | NS5 | 9705 | T | TC | Phe713fs | 1.07 |
| NS2A | 3928 | G | C | Ala151Pro | 1.58 | NS5 | 9714 | G | C | Glu715Asp | 0.72 |
| NS2B | 4161 | A | T | - | 0.61 | NS5 | 9716 | T | A | Leu716* | 0.69 |
| NS2B | 4188 | T | C | - | 0.61 | NS5 | 9718 | G | C | Val717Leu | 0.72 |
| NS3 | 4581 | T | C | - | 2.22 | NS5 | 9726 | AG | A | Asp720fs | 1.06 |
| NS3 | 4635 | C | T | - | 0.59 | NS5 | 9877 | C | T | Arg770Cys | 0.76 |
| NS3 | 5026 | G | GA | Ser171fs | 6.88 | NS5 | 9902 | C | G | Ala778Gly | 2.80 |
| NS3 | 5136 | A | C | - | 0.67 | NS5 | 9969 | G | A | - | 0.82 |
| NS3 | 5304 | C | G | Cys261Trp | 1.54 | 3UTR | 10389 | CA | C | - | 7.40 |
| NS3 | 5309 | C | G | Ala263Gly | 0.90 | 3UTR | 10600 | A | AC | - | 0.65 |

| **170** | | | | | | NS3 | 5026 | G | GA | Ser171fs | 6.28 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | | | | | | NS3 | 5135 | C | A | Pro205Gln | 0.62 |

| Primary | | | | | |
|---|---|---|---|---|---|
| 5.17E+04 | | | | | |
| 1 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 111 | G | A | - | 1.59 |
| C | 135 | C | T | - | 5.56 |
| C | 341 | G | T | Arg82Ile | 2.24 |
| prM/M | 450 | C | T | - | 0.75 |
| prM/M | 552 | C | T | - | 0.58 |
| prM/M | 703 | G | GA | Arg91fs | 2.75 |
| prM/M | 765 | A | C | - | 0.68 |
| prM/M | 855 | T | C | - | 1.29 |
| prM/M | 885 | C | T | - | 1.14 |
| E | 954 | A | C | - | 1.08 |
| E | 1094 | C | T | Pro53Leu | 0.90 |
| E | 1095 | C | T | - | 0.92 |
| E | 1313 | A | C | Glu126Ala | 1.54 |
| E | 1595 | G | T | Trp220Leu | 1.51 |
| E | 1695 | A | G | - | 1.39 |
| E | 1994 | C | CGATTG | Val354fs | 2.40 |
| E | 2210 | T | A | Leu425* | 1.51 |
| E | 2303 | A | G | Lys456Arg | 1.37 |
| E | 2308 | C | T | Leu458Phe | 1.64 |
| E | 2315 | G | T | Gly460Val | 1.06 |
| NS1 | 2448 | A | G | - | 1.04 |
| NS1 | 2484 | C | T | - | 1.01 |
| NS1 | 3043 | G | A | Asp208Asn | 0.57 |
| NS1 | 3152 | C | CA | Asn246fs | 7.00 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 1.86 |
| NS2A | 3489 | G | A | - | 0.88 |
| NS2A | 3624 | T | C | - | 0.86 |
| NS2A | 3627 | T | A | Phe50Leu | 1.59 |
| NS2A | 3634 | C | A | Leu53Met | 0.72 |
| NS2A | 3716 | C | T | Ala80Val | 0.97 |
| NS2A | 3720 | C | CA | Val83fs | 1.39 |
| NS2A | 3866 | G | C | Gly130Ala | 1.65 |
| NS2A | 3928 | G | C | Ala151Pro | 1.37 |
| NS2B | 4161 | A | T | - | 0.71 |
| NS2B | 4341 | T | C | - | 0.98 |
| NS2B | 4482 | G | A | - | 0.65 |
| NS3 | 4908 | T | C | - | 0.74 |
| NS3 | 5026 | G | A | Glu169Lys | 1.07 |
| NS3 | 5304 | T | G | Cys261Trp | 1.03 |
| NS3 | 5309 | C | A | Ala263Asp | 0.92 |
| NS3 | 5433 | A | T | - | 1.23 |
| NS3 | 5436 | G | A | - | 1.47 |
| NS3 | 5447 | C | T | Ala309Val | 0.53 |
| NS3 | 5531 | G | T | Arg337Ile | 0.97 |
| NS3 | 5575 | A | T | Thr352Ser | 1.20 |
| NS3 | 5754 | A | T | - | 0.50 |
| NS3 | 5839 | C | T | Arg440Trp | 2.45 |
| NS3 | 5915 | C | CA | Asn467fs | 6.56 |
| NS3 | 6078 | A | T | - | 2.91 |
| NS3 | 6105 | G | A | - | 9.47 |
| NS3 | 6285 | G | GA | Lys590fs | 3.00 |
| NS3 | 6365 | C | T | Ala615Val | 2.17 |
| NS4A | 6711 | G | GT | Leu115fs | 5.36 |
| NS4A | 6790 | A | T | Ile139Leu | 0.82 |
| NS4B | 6844 | G | A | Glu7Lys | 1.32 |
| NS4B | 6913 | A | G | Ile30Val | 1.09 |
| NS4B | 6922 | C | T | Arg33Cys | 1.54 |
| NS4B | 6958 | A | G | Thr45Ala | 0.84 |
| NS4B | 6982 | C | T | Arg53* | 1.50 |
| NS4B | 7428 | C | T | - | 0.55 |
| NS4B | 7435 | C | G | Leu204Val | 0.90 |
| NS5 | 7640 | A | C | Glu24Ala | 1.02 |
| NS5 | 7645 | C | A | Gln26Lys | 1.13 |
| NS5 | 7920 | A | C | - | 0.56 |
| NS5 | 8074 | G | C | Glu169Gln | 1.54 |
| NS5 | 8184 | G | C | Leu205Phe | 0.65 |
| NS5 | 8306 | A | C | Lys246Thr | 0.96 |
| NS5 | 8361 | C | T | - | 3.92 |
| NS5 | 8499 | A | T | Glu310Asp | 0.85 |
| NS5 | 8891 | T | TA | His442fs | 1.20 |
| NS5 | 8926 | A | AC | Asn453fs | 2.37 |
| NS5 | 8932 | A | T | Met455Leu | 1.15 |
| NS5 | 9576 | T | C | - | 0.87 |
| NS5 | 9705 | T | TC | Phe713fs | 1.26 |
| NS5 | 9795 | G | A | - | 0.89 |
| NS5 | 9902 | C | G | Ala778Gly | 2.78 |
| NS5 | 9942 | C | T | - | 0.71 |
| 3UTR | 10389 | CAA | C | - | 7.20 |
| 3UTR | 10502 | C | A | - | 0.75 |
| 3UTR | 10613 | C | CA | - | 2.85 |

| **171** | | | | | |
|---|---|---|---|---|---|
| DF | | | | | |
| Primary | | | | | |
| 1.35E+06 | | | | | |
| 1 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 70 | G | GT | - | 2.17 |
| 5UTR | 96 | G | A | - | 1.44 |
| C | 110 | G | GA | Lys6fs | 1.80 |
| C | 135 | C | T | - | 1.18 |
| C | 144 | G | GA | Arg18fs | 0.59 |
| C | 312 | C | CA | Ser75fs | 3.49 |
| C | 341 | G | T | Arg82Ile | 3.22 |
| prM/M | 457 | A | C | Asn7His | 0.80 |
| prM/M | 666 | T | C | - | 1.23 |
| prM/M | 703 | G | GA | Arg91fs | 2.96 |
| NS3 | 5717 | C | T | Ala399Val | 0.84 |
| NS3 | 5722 | G | C | Asp401His | 0.84 |
| NS3 | 5754 | A | T | - | 0.51 |
| NS3 | 5839 | C | T | Arg440Trp | 1.87 |
| NS3 | 5915 | C | CA | Asn467fs | 7.00 |
| NS3 | 6285 | G | A | - | 2.35 |
| NS3 | 6285 | G | GA | Lys590fs | 4.26 |
| NS3 | 6365 | C | T | Ala615Val | 1.61 |
| NS4A | 6655 | C | T | Leu94Phe | 0.76 |
| NS4A | 6711 | G | GT | Leu115fs | 5.85 |
| NS4A | 6790 | A | T | Ile139Leu | 0.99 |
| NS4B | 6844 | G | A | Glu7Lys | 1.03 |
| NS4B | 6913 | A | G | Ile30Val | 0.89 |
| NS4B | 6922 | C | T | Arg33Cys | 1.65 |
| NS4B | 6958 | A | G | Thr45Ala | 0.88 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| prM/M | 734 | T | TGGAACGAGC | Val99Gly100insGluGlnAla | 0.62 | NS4B | 6982 | C | T | Arg53* | 1.68 |
| E | 1040 | C | CA | Asn37fs | 7.42 | NS4B | 7049 | T | C | Leu75Ser | 0.51 |
| E | 1311 | G | A | Met125Ile | 1.26 | NS4B | 7158 | T | G | - | 1.30 |
| E | 1595 | G | T | Trp220Leu | 1.28 | NS4B | 7435 | C | G | Leu204Val | 0.58 |
| E | 1695 | A | G | - | 0.92 | NS4B | 7471 | A | G | Thr216Ala | 0.87 |
| E | 2210 | T | A | Leu425* | 1.05 | NS5 | 7645 | C | A | Gln26Lys | 1.69 |
| E | 2308 | C | T | Leu458Phe | 1.22 | NS5 | 7784 | C | T | Thr72Ile | 1.73 |
| NS1 | 3152 | C | CA | Asn246fs | 7.80 | NS5 | 7856 | A | G | Asn96Ser | 1.18 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 2.95 | NS5 | 8074 | G | C | Glu169Gln | 1.94 |
| NS1 | 3398 | A | G | Glu326Gly | 0.90 | NS5 | 8184 | G | C | Leu205Phe | 0.75 |
| NS2A | 3716 | C | T | Ala80Val | 0.62 | NS5 | 8204 | G | C | Arg212Pro | 0.86 |
| NS2A | 3720 | C | CA | Val83fs | 1.80 | NS5 | 8571 | C | T | - | 0.80 |
| NS2A | 3866 | G | C | Gly130Ala | 1.85 | NS5 | 8745 | GA | G | Met393fs | 1.93 |
| NS2A | 3928 | G | C | Ala151Pro | 1.71 | NS5 | 8891 | T | TA | His442fs | 2.06 |
| NS2B | 4263 | C | T | - | 3.44 | NS5 | 8926 | A | AC | Asn453fs | 5.81 |
| NS2B | 4388 | T | TA | Asn88fs | 0.97 | NS5 | 8932 | A | T | Met455Leu | 1.90 |
| NS3 | 5026 | G | GA | Ser171fs | 6.21 | NS5 | 9705 | T | TC | Phe713fs | 0.86 |
| NS3 | 5304 | C | G | Cys261Trp | 1.32 | NS5 | 9814 | A | G | Lys749Glu | 20.32 |
| NS3 | 5309 | C | G | Ala263Gly | 1.16 | NS5 | 9902 | C | G | Ala778Gly | 2.58 |
| NS3 | 5433 | A | T | - | 1.28 | NS5 | 9966 | CA | C | Lys800fs | 1.34 |
| NS3 | 5436 | G | A | - | 1.70 | 3UTR | 10278 | G | T | - | 28.73 |
| NS3 | 5531 | G | T | Arg337Ile | 1.02 | 3UTR | 10612 | C | CA | - | 1.95 |

| **172** | | | | | |
|---|---|---|---|---|---|
| DF | | | | | |
| Primary | | | | | |
| 8.38E+06 | | | | | |
| 2 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 96 | G | A | - | 1.82 |
| C | 135 | C | T | - | 2.95 |
| C | 312 | C | CA | Ser75fs | 2.27 |
| C | 341 | G | T | Arg82Ile | 4.48 |
| prM/M | 457 | A | C | Asn7His | 0.79 |
| prM/M | 703 | G | GA | Arg91fs | 1.46 |
| E | 1287 | T | C | - | 0.78 |
| E | 1311 | G | A | Met125Ile | 1.46 |
| E | 1595 | G | T | Trp220Leu | 1.43 |
| E | 1687 | G | A | Val251Ile | 0.55 |
| E | 1689 | T | C | - | 0.91 |
| E | 1695 | A | G | - | 1.66 |
| E | 1731 | A | G | - | 1.24 |
| E | 1994 | C | CGATTG | Val354fs | 1.24 |
| E | 2108 | G | GA | Trp391fs | 0.49 |
| E | 2315 | G | T | Gly460Val | 0.85 |
| E | 2411 | T | C | Met492Thr | 0.52 |
| NS1 | 2907 | T | C | - | 0.96 |
| NS1 | 3001 | G | A | Val194Ile | 1.86 |
| NS1 | 3053 | A | C | Lys211Thr | 0.82 |
| NS1 | 3398 | A | G | Glu326Gly | 1.13 |
| NS2A | 3503 | C | A | Ser9* | 0.62 |
| NS2A | 3716 | C | T | Ala80Val | 0.68 |
| NS2A | 3720 | C | CA | Val83fs | 1.39 |
| NS2A | 3928 | G | C | Ala151Pro | 1.41 |
| NS2A | 4089 | A | T | - | 0.55 |
| NS3 | 4837 | C | T | Pro106Ser | 0.75 |
| NS3 | 4843 | G | A | Ala108Thr | 0.72 |
| NS3 | 4863 | A | G | - | 0.90 |
| NS3 | 5133 | T | C | - | 1.18 |
| NS3 | 5717 | C | T | Ala399Val | 1.38 |
| NS3 | 5722 | G | C | Asp401His | 0.50 |
| NS3 | 5839 | C | T | Arg440Trp | 2.53 |
| NS3 | 5915 | C | CA | Asn467fs | 3.34 |
| NS3 | 6074 | C | T | - | 1.29 |
| NS3 | 6155 | G | A | Trp545* | 1.05 |
| NS3 | 6285 | G | A | - | 0.89 |
| NS3 | 6285 | G | GA | Lys590fs | 1.96 |
| NS3 | 6365 | C | T | Ala615Val | 1.39 |
| NS4A | 6377 | C | T | Ser1Phe | 0.51 |
| NS4A | 6614 | A | G | Lys80Arg | 0.59 |
| NS4A | 6655 | C | T | Leu94Phe | 1.01 |
| NS4A | 6711 | G | GT | Leu115fs | 2.15 |
| NS4A | 6790 | A | T | Ile139Leu | 1.50 |
| NS4B | 6844 | G | A | Glu7Lys | 0.94 |
| NS4B | 6982 | C | T | Arg53* | 1.53 |
| NS4B | 7049 | T | C | Leu75Ser | 0.84 |
| NS4B | 7071 | G | T | Trp82Cys | 0.54 |
| NS4B | 7158 | T | G | - | 1.68 |
| NS4B | 7471 | A | G | Thr216Ala | 0.66 |
| NS5 | 7619 | T | G | Leu17Trp | 0.75 |
| NS5 | 7645 | C | A | Gln26Lys | 0.98 |
| NS5 | 7656 | G | A | - | 0.51 |
| NS5 | 7784 | C | T | Thr72Ile | 0.84 |
| NS5 | 7920 | A | C | - | 0.70 |
| NS5 | 8074 | G | C | Glu169Gln | 2.17 |
| NS5 | 8175 | A | G | - | 0.79 |
| NS5 | 8184 | G | C | Leu205Phe | 1.50 |
| NS5 | 8258 | C | A | Ser230* | 0.76 |
| NS5 | 8571 | C | T | - | 0.49 |
| NS5 | 8848 | G | C | Glu427Gln | 0.56 |
| NS5 | 8891 | T | TA | His442fs | 1.22 |
| NS5 | 8919 | TG | T | Val451fs | 1.44 |
| NS5 | 8926 | A | AC | Asn453fs | 3.69 |
| NS5 | 9029 | A | C | Glu487Ala | 0.88 |
| NS5 | 9207 | C | T | - | 12.05 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NS3 | 5135 | C | A | Pro205Gln | 0.50 | NS5 | 9545 | C | T | Ala659Val | 0.63 |
| NS3 | 5136 | A | T | - | 0.96 | NS5 | 9617 | A | G | Asp683Gly | 0.86 |
| NS3 | 5188 | C | T | Pro223Ser | 0.79 | NS5 | 9705 | T | TC | Phe713fs | 1.18 |
| NS3 | 5304 | C | G | Cys261Trp | 1.37 | NS5 | 9714 | G | C | Glu715Asp | 1.01 |
| NS3 | 5436 | G | A | - | 2.04 | NS5 | 9716 | T | A | Leu716* | 1.02 |
| NS3 | 5521 | G | A | Asp334Asn | 0.67 | NS5 | 9718 | G | C | Val717Leu | 1.02 |
| NS3 | 5531 | G | T | Arg337Ile | 1.39 | NS5 | 9726 | AG | A | Asp720fs | 1.20 |
| NS3 | 5543 | A | C | Glu341Ala | 1.40 | NS5 | 9902 | C | G | Ala778Gly | 0.92 |
| NS3 | 5575 | A | T | Thr352Ser | 0.68 | 3UTR | 10501 | C | A | - | 0.56 |
| NS3 | 5642 | G | T | Cys374Phe | 0.64 | 3UTR | 10630 | C | T | - | 0.65 |

| **173** | | | | | | NS3 | 5289 | C | A | - | 3.21 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | | | | | | NS3 | 5304 | T | G | Cys261Trp | 1.78 |
| Secondary | | | | | | NS3 | 5309 | C | A | Ala263Asp | 1.75 |
| 2.82E+04 | | | | | | NS3 | 5436 | G | A | - | 0.72 |
| 4 | | | | | | NS3 | 5543 | A | C | Glu341Ala | 1.02 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5839 | C | T | Arg440Trp | 2.50 |
| 5UTR | 70 | G | GT | - | 1.22 | NS3 | 5844 | A | G | - | 0.95 |
| C | 135 | C | T | - | 1.28 | NS3 | 5915 | C | CA | Asn467fs | 2.69 |
| C | 341 | G | T | Arg82Ile | 1.85 | NS3 | 5979 | C | A | - | 0.88 |
| C | 411 | T | C | - | 0.51 | NS3 | 6042 | T | C | - | 1.69 |
| prM/M | 703 | G | GA | Arg91fs | 3.32 | NS4A | 6606 | T | C | - | 0.79 |
| prM/M | 834 | C | T | - | 0.76 | NS4A | 6655 | C | T | Leu94Phe | 0.54 |
| E | 1040 | C | CA | Asn37fs | 3.87 | NS4A | 6711 | G | GT | Leu115fs | 1.64 |
| E | 1094 | C | T | Pro53Leu | 0.60 | NS4A | 6790 | A | T | Ile139Leu | 1.14 |
| E | 1287 | T | C | - | 1.09 | NS4B | 6844 | G | A | Glu7Lys | 0.51 |
| E | 1302 | A | G | - | 1.16 | NS4B | 6922 | C | T | Arg33Cys | 1.22 |
| E | 1311 | G | A | Met125Ile | 0.86 | NS4B | 6958 | A | G | Thr45Ala | 0.70 |
| E | 1353 | T | C | - | 0.89 | NS4B | 6982 | C | T | Arg53* | 1.21 |
| E | 1595 | G | T | Trp220Leu | 1.08 | NS4B | 7158 | T | G | - | 1.43 |
| E | 1695 | A | G | - | 0.80 | NS5 | 7640 | A | C | Glu24Ala | 1.44 |
| E | 1986 | C | T | - | 0.94 | NS5 | 7645 | C | A | Gln26Lys | 1.28 |
| E | 1994 | C | CGATTG | Val354fs | 0.85 | NS5 | 7656 | G | A | - | 2.50 |
| E | 2055 | T | C | - | 0.90 | NS5 | 7763 | T | C | Phe65Ser | 3.08 |
| E | 2210 | T | A | Leu425* | 1.02 | NS5 | 8074 | G | C | Glu169Gln | 1.60 |
| E | 2244 | C | T | - | 0.75 | NS5 | 8204 | G | C | Arg212Pro | 0.55 |
| E | 2303 | A | G | Lys456Arg | 1.58 | NS5 | 8569 | A | G | Ile334Val | 0.94 |
| E | 2308 | C | T | Leu458Phe | 1.75 | NS5 | 8602 | G | A | Asp345Asn | 1.41 |
| E | 2315 | G | T | Gly460Val | 1.19 | NS5 | 8926 | A | AC | Asn453fs | 2.18 |
| NS1 | 2578 | G | A | Gly53Ser | 1.48 | NS5 | 9029 | A | C | Glu487Ala | 0.70 |
| NS1 | 3152 | C | CA | Asn246fs | 3.91 | NS5 | 9705 | T | TC | Phe713fs | 1.33 |
| NS1 | 3160 | T | G | Leu247Val | 1.44 | NS5 | 9714 | G | C | Glu715Asp | 0.74 |
| NS1 | 3166 | G | T | Gly249* | 2.40 | NS5 | 9718 | G | C | Val717Leu | 0.63 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 3.15 | NS5 | 9726 | AG | A | Asp720fs | 1.11 |
| NS1 | 3398 | A | G | Glu326Gly | 0.93 | NS5 | 9877 | C | T | Arg770Cys | 1.05 |
| NS2A | 3866 | G | C | Gly130Ala | 1.31 | NS5 | 9902 | C | G | Ala778Gly | 4.02 |
| NS2A | 3928 | G | C | Ala151Pro | 1.11 | 3UTR | 10389 | CAA | C | - | 4.20 |
| NS2B | 4188 | T | C | - | 0.69 | 3UTR | 10445 | T | TA | - | 1.96 |
| NS3 | 5026 | G | GA | Ser171fs | 2.66 | 3UTR | 10613 | C | CA | - | 0.63 |

| **174** | | | | | | NS3 | 5309 | C | G | Ala263Gly | 1.07 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | | | | | | NS3 | 5433 | A | T | - | 1.25 |
| Primary | | | | | | NS3 | 5436 | G | A | - | 1.32 |
| 2.69E+04 | | | | | | NS3 | 5521 | G | A | Asp334Asn | 0.87 |
| 3 | | | | | | NS3 | 5530 | A | AT | Arg337fs | 0.52 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5531 | G | T | Arg337Ile | 0.92 |
| 5UTR | 70 | G | GT | - | 0.82 | NS3 | 5839 | C | T | Arg440Trp | 1.33 |
| 5UTR | 96 | G | A | - | 1.42 | NS3 | 5915 | C | CA | Asn467fs | 5.21 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 110 | G | GA | Lys6fs | 0.69 | NS3 | 6155 | G | A | Trp545* | 1.17 |
| C | 135 | C | T | - | 2.86 | NS3 | 6285 | G | A | - | 1.35 |
| C | 201 | C | T | - | 1.00 | NS3 | 6285 | G | GA | Lys590fs | 2.00 |
| C | 231 | G | A | - | 0.84 | NS3 | 6365 | C | T | Ala615Val | 1.34 |
| C | 387 | A | G | - | 1.19 | NS4A | 6378 | C | T | - | 0.91 |
| C | 393 | C | T | - | 1.64 | NS4A | 6379 | C | T | - | 1.02 |
| C | 431 | C | T | Ala112Val | 0.82 | NS4A | 6418 | A | G | Thr15Ala | 0.91 |
| prM/M | 445 | C | T | - | 1.11 | NS4A | 6462 | C | T | - | 1.22 |
| prM/M | 450 | T | C | - | 1.40 | NS4A | 6491 | G | A | Arg39Lys | 1.10 |
| prM/M | 457 | A | C | Asn7His | 1.48 | NS4A | 6496 | C | T | His41Tyr | 0.91 |
| prM/M | 703 | G | GA | Arg91fs | 1.62 | NS4A | 6547 | T | C | - | 0.96 |
| prM/M | 717 | G | A | - | 1.75 | NS4A | 6562 | A | G | Thr63Ala | 0.84 |
| prM/M | 834 | C | T | - | 0.95 | NS4B | 6844 | G | GA | Thr9fs | 1.11 |
| prM/M | 837 | C | T | - | 0.52 | NS4B | 6844 | G | A | Glu7Lys | 1.01 |
| E | 941 | G | C | Arg2Pro | 0.82 | NS4B | 6864 | C | T | - | 0.60 |
| E | 954 | C | A | - | 0.76 | NS4B | 6879 | C | T | - | 0.73 |
| E | 1005 | A | C | - | 0.82 | NS4B | 6922 | C | T | Arg33Cys | 1.02 |
| E | 1311 | G | A | Met125Ile | 1.70 | NS4B | 6982 | C | T | Arg53* | 0.78 |
| E | 1590 | A | G | - | 0.96 | NS4B | 7041 | C | T | - | 0.56 |
| E | 1595 | G | T | Trp220Leu | 1.04 | NS4B | 7049 | T | C | Leu75Ser | 0.79 |
| E | 1641 | A | G | - | 0.86 | NS4B | 7371 | A | G | - | 0.85 |
| E | 1689 | C | T | - | 1.66 | NS5 | 7962 | C | T | - | 2.26 |
| E | 1994 | C | CGATTG | Val354fs | 0.75 | NS5 | 8074 | G | C | Glu169Gln | 1.49 |
| E | 2210 | T | A | Leu425* | 0.95 | NS5 | 8235 | C | T | - | 0.66 |
| E | 2223 | C | T | - | 0.58 | NS5 | 8382 | A | G | Ile271Met | 5.03 |
| E | 2308 | C | T | Leu458Phe | 0.98 | NS5 | 8602 | G | A | Asp345Asn | 1.97 |
| NS1 | 2484 | C | T | - | 0.61 | NS5 | 8891 | T | TA | His442fs | 0.74 |
| NS1 | 3152 | C | CA | Asn246fs | 5.45 | NS5 | 8919 | TG | T | Val451fs | 0.51 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 1.99 | NS5 | 8926 | A | AC | Asn453fs | 1.79 |
| NS1 | 3398 | A | G | Glu326Gly | 1.21 | NS5 | 9576 | C | T | - | 0.64 |
| NS1 | 3402 | C | T | - | 0.56 | NS5 | 9705 | T | TC | Phe713fs | 0.78 |
| NS2A | 3858 | G | A | - | 0.70 | NS5 | 9726 | AG | A | Asp720fs | 0.61 |
| NS2A | 3866 | G | C | Gly130Ala | 1.15 | NS5 | 9813 | G | C | Leu748Phe | 0.50 |
| NS2A | 3928 | G | C | Ala151Pro | 1.03 | NS5 | 9902 | C | G | Ala778Gly | 3.78 |
| NS3 | 5304 | C | G | Cys261Trp | 1.64 | 3UTR | 10408 | C | T | - | 1.02 |
| NS3 | 5304 | C | T | - | 1.64 | 3UTR | 10501 | C | A | - | 0.58 |

| **175** | | | | | | NS3 | 5748 | C | T | - | 10.80 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | | | | | | NS3 | 5839 | C | T | Arg440Trp | 1.35 |
| Secondary | | | | | | NS3 | 5915 | C | CA | Asn467fs | 3.62 |
| 9.48E+02 | | | | | | NS3 | 6076 | A | G | Ile519Val | 4.07 |
| 1 | | | | | | NS3 | 6094 | C | T | - | 2.01 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 6105 | G | A | - | 2.76 |
| C | 135 | C | T | - | 1.72 | NS3 | 6285 | G | GA | Lys590fs | 1.42 |
| C | 312 | C | CA | Ser75fs | 3.30 | NS3 | 6365 | C | T | Ala615Val | 1.23 |
| C | 341 | G | T | Arg82Ile | 3.38 | NS4A | 6528 | C | T | - | 0.67 |
| prM/M | 457 | A | C | Asn7His | 0.68 | NS4A | 6633 | C | T | - | 0.51 |
| prM/M | 703 | G | GA | Arg91fs | 2.95 | NS4A | 6655 | C | T | Leu94Phe | 0.67 |
| prM/M | 765 | A | C | - | 0.69 | NS4A | 6790 | A | T | Ile139Leu | 1.27 |
| prM/M | 834 | C | T | - | 0.99 | NS4B | 6844 | G | A | Glu7Lys | 1.29 |
| E | 1311 | G | A | Met125Ile | 1.49 | NS4B | 6876 | C | T | - | 0.58 |
| E | 1595 | G | T | Trp220Leu | 1.66 | NS4B | 6922 | C | T | Arg33Cys | 1.25 |
| E | 1695 | A | G | - | 0.84 | NS4B | 6958 | A | G | Thr45Ala | 0.73 |
| E | 1994 | C | CGATTG | Val354fs | 1.20 | NS4B | 6982 | C | T | Arg53* | 1.29 |
| E | 2145 | G | A | - | 7.89 | NS4B | 7049 | T | C | Leu75Ser | 0.53 |
| E | 2210 | T | A | Leu425* | 0.82 | NS4B | 7158 | T | G | - | 1.12 |
| E | 2269 | G | A | Gly445Arg | 0.54 | NS4B | 7435 | C | G | Leu204Val | 0.52 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 2296 | A | T | Thr454Ser | 0.81 | NS5 | 7645 | C | A | Gln26Lys | 1.98 |
| E | 2303 | A | G | Lys456Arg | 1.60 | NS5 | 7763 | T | C | Phe65Ser | 2.98 |
| E | 2308 | C | T | Leu458Phe | 1.73 | NS5 | 8074 | G | C | Glu169Gln | 1.13 |
| E | 2315 | G | T | Gly460Val | 1.38 | NS5 | 8602 | G | A | Asp345Asn | 1.63 |
| NS1 | 2580 | C | A | - | 1.11 | NS5 | 8891 | T | TA | His442fs | 0.94 |
| NS1 | 3160 | T | G | Leu247Val | 1.13 | NS5 | 8926 | A | AC | Asn453fs | 2.97 |
| NS1 | 3166 | G | T | Gly249* | 2.78 | NS5 | 8932 | A | T | Met455Leu | 1.39 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 1.93 | NS5 | 9006 | T | C | - | 0.77 |
| NS1 | 3329 | C | T | Ala303Val | 0.50 | NS5 | 9705 | T | TC | Phe713fs | 0.95 |
| NS2A | 3716 | C | T | Ala80Val | 0.56 | NS5 | 9714 | G | C | Glu715Asp | 0.53 |
| NS2A | 3866 | G | C | Gly130Ala | 0.99 | NS5 | 9726 | AG | A | Asp720fs | 0.71 |
| NS2B | 4188 | T | C | - | 0.61 | NS5 | 9737 | T | A | Val723Glu | 1.24 |
| NS3 | 4826 | C | T | Pro102Leu | 0.51 | NS5 | 9783 | C | A | - | 0.51 |
| NS3 | 4863 | A | G | - | 1.50 | NS5 | 9877 | C | T | Arg770Cys | 0.89 |
| NS3 | 5136 | A | T | - | 0.74 | NS5 | 9902 | C | G | Ala778Gly | 3.55 |
| NS3 | 5433 | A | T | - | 0.86 | NS5 | 10110 | C | T | - | 1.26 |
| NS3 | 5436 | G | A | - | 1.19 | 3UTR | 10401 | A | G | - | 2.07 |
| NS3 | 5531 | G | T | Arg337Ile | 0.55 | 3UTR | 10613 | C | CAA | - | 0.60 |

| **177** | | | | | | NS3 | 5457 | T | TTCTCTTTCTTCA | Ile312Phe313SerLeuSerSer | 0.75 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | | | | | | NS3 | 5521 | G | A | Asp334Asn | 0.65 |
| Primary | | | | | | NS3 | 5526 | A | G | - | 5.56 |
| 3.18E+04 | | | | | | NS3 | 5543 | A | C | Glu341Ala | 1.26 |
| 4 | | | | | | NS3 | 5642 | G | T | Cys374Phe | 0.76 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5838 | G | A | - | 5.16 |
| 5UTR | 70 | G | GT | - | 0.56 | NS3 | 5839 | C | T | Arg440Trp | 1.80 |
| 5UTR | 96 | G | A | - | 1.20 | NS3 | 5915 | C | CA | Asn467fs | 3.93 |
| C | 135 | C | T | - | 2.40 | NS3 | 6074 | C | T | - | 0.87 |
| C | 341 | G | T | Arg82Ile | 1.81 | NS3 | 6285 | G | A | - | 0.82 |
| prM/M | 457 | A | C | Asn7His | 1.22 | NS3 | 6285 | G | GA | Lys590fs | 2.16 |
| prM/M | 606 | C | T | - | 0.69 | NS3 | 6365 | C | T | Ala615Val | 0.87 |
| prM/M | 645 | C | T | - | 0.69 | NS4A | 6418 | A | G | Thr15Ala | 1.11 |
| prM/M | 703 | G | GA | Arg91fs | 1.92 | NS4A | 6599 | GA | G | Arg76fs | 0.49 |
| prM/M | 834 | C | T | - | 0.64 | NS4A | 6711 | G | GT | Leu115fs | 1.73 |
| E | 941 | G | C | Arg2Pro | 1.09 | NS4A | 6790 | A | T | Ile139Leu | 1.26 |
| E | 1287 | T | C | - | 1.00 | NS4B | 6844 | G | A | Glu7Lys | 1.03 |
| E | 1311 | G | A | Met125Ile | 1.78 | NS4B | 6922 | C | T | Arg33Cys | 1.37 |
| E | 1390 | G | C | Gly152Arg | 0.70 | NS4B | 6982 | C | T | Arg53* | 0.87 |
| E | 1595 | G | T | Trp220Leu | 1.45 | NS4B | 7049 | T | C | Leu75Ser | 0.74 |
| E | 1689 | C | T | - | 0.81 | NS4B | 7158 | T | G | - | 1.05 |
| E | 1780 | C | T | His282Tyr | 6.72 | NS5 | 7645 | C | A | Gln26Lys | 1.21 |
| E | 1911 | G | A | - | 0.93 | NS5 | 7920 | A | C | - | 0.82 |
| E | 1994 | C | CGATTG | Val354fs | 0.69 | NS5 | 8074 | G | C | Glu169Gln | 1.39 |
| E | 2012 | C | T | Thr359Ile | 0.62 | NS5 | 8214 | A | G | - | 0.58 |
| E | 2103 | C | A | - | 0.55 | NS5 | 8258 | C | A | Ser230* | 0.88 |
| E | 2210 | T | A | Leu425* | 0.61 | NS5 | 8370 | T | A | - | 1.78 |
| E | 2308 | C | T | Leu458Phe | 0.59 | NS5 | 8499 | A | T | Glu310Asp | 1.92 |
| NS1 | 2665 | A | T | Asn82Tyr | 0.58 | NS5 | 8602 | G | A | Asp345Asn | 0.83 |
| NS1 | 2760 | C | T | - | 0.74 | NS5 | 8891 | T | TA | His442fs | 1.09 |
| NS1 | 2791 | T | C | Phe124Leu | 0.57 | NS5 | 8926 | A | AC | Asn453fs | 2.17 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 2.17 | NS5 | 8932 | A | T | Met455Leu | 1.57 |
| NS2A | 3858 | G | A | - | 0.49 | NS5 | 9705 | T | TC | Phe713fs | 1.02 |
| NS2A | 3866 | G | C | Gly130Ala | 1.08 | NS5 | 9714 | G | C | Glu715Asp | 0.74 |
| NS2B | 4167 | G | A | - | 0.80 | NS5 | 9716 | T | A | Leu716* | 0.77 |
| NS3 | 4533 | G | A | - | 1.98 | NS5 | 9718 | G | C | Val717Leu | 0.76 |
| NS3 | 4826 | C | T | Pro102Leu | 0.60 | NS5 | 9726 | AG | A | Asp720fs | 0.93 |
| NS3 | 5026 | G | A | Glu169Lys | 0.76 | NS5 | 9810 | C | T | - | 0.70 |
| NS3 | 5188 | C | T | Pro223Ser | 0.82 | NS5 | 9813 | G | C | Leu748Phe | 0.50 |
| NS3 | 5304 | C | G | Cys261Trp | 1.12 | NS5 | 9877 | C | T | Arg770Cys | 0.56 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS3 | 5309 | C | G | Ala263Gly | 0.87 | NS5 | 9902 | C | G | Ala778Gly | 2.68 |
| NS3 | 5409 | A | T | - | 1.02 | NS5 | 10083 | T | C | - | 0.73 |
| NS3 | 5433 | A | T | - | 1.20 | NS5 | 10163 | C | T | Ala865Val | 1.30 |
| NS3 | 5436 | G | A | - | 1.37 | NS5 | 10261 | A | G | Ile898Val | 1.48 |

| **178** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | | | | | | NS3 | 5531 | G | T | Arg337Ile | 1.57 |
| Primary | | | | | | NS3 | 5717 | C | T | Ala399Val | 0.82 |
| 4.98E+04 | | | | | | NS3 | 5754 | A | T | - | 0.66 |
| 4 | | | | | | NS3 | 5839 | C | T | Arg440Trp | 2.31 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5915 | C | CA | Asn467fs | 5.29 |
| 5UTR | 70 | G | GT | - | 1.21 | NS3 | 6365 | C | T | Ala615Val | 1.18 |
| 5UTR | 96 | G | A | - | 1.57 | NS4A | 6711 | G | GT | Leu115fs | 3.10 |
| C | 110 | G | GA | Lys6fs | 0.64 | NS4A | 6790 | A | T | Ile139Leu | 1.21 |
| C | 135 | C | T | - | 3.47 | NS4B | 6844 | G | GA | Thr9fs | 0.89 |
| C | 181 | C | T | - | 1.06 | NS4B | 6844 | G | A | Glu7Lys | 1.29 |
| C | 341 | G | T | Arg82Ile | 1.20 | NS4B | 6913 | A | G | Ile30Val | 0.95 |
| prM/M | 457 | A | C | Asn7His | 1.43 | NS4B | 6922 | C | T | Arg33Cys | 1.39 |
| prM/M | 703 | G | GA | Arg91fs | 4.62 | NS4B | 6982 | C | T | Arg53* | 1.01 |
| prM/M | 765 | A | C | - | 0.79 | NS4B | 7049 | T | C | Leu75Ser | 0.66 |
| E | 1287 | T | C | - | 1.37 | NS5 | 7645 | C | A | Gln26Lys | 1.05 |
| E | 1311 | G | A | Met125Ile | 2.00 | NS5 | 8074 | G | C | Glu169Gln | 1.62 |
| E | 1595 | G | T | Trp220Leu | 1.08 | NS5 | 8175 | A | G | - | 0.88 |
| E | 1681 | G | A | Asp249Asn | 1.10 | NS5 | 8258 | C | A | Ser230* | 0.96 |
| E | 1687 | G | A | Val251Ile | 0.65 | NS5 | 8602 | G | A | Asp345Asn | 1.55 |
| E | 1994 | C | CGATTG | Val354fs | 0.84 | NS5 | 8891 | T | TA | His442fs | 1.75 |
| E | 2308 | C | T | Leu458Phe | 0.97 | NS5 | 8919 | TG | T | Val451fs | 0.57 |
| NS1 | 3152 | C | CA | Asn246fs | 6.57 | NS5 | 8926 | A | AC | Asn453fs | 2.10 |
| NS1 | 3241 | G | GCCT | Glu274delinsAlaTer | 1.89 | NS5 | 8932 | A | T | Met455Leu | 1.21 |
| NS2A | 3598 | G | A | Val41Met | 1.08 | NS5 | 9705 | T | TC | Phe713fs | 0.84 |
| NS2A | 3866 | G | C | Gly130Ala | 1.02 | NS5 | 9714 | G | C | Glu715Asp | 0.71 |
| NS2A | 3928 | G | C | Ala151Pro | 1.09 | NS5 | 9718 | G | C | Val717Leu | 0.68 |
| NS3 | 4562 | G | A | Gly14Glu | 2.46 | NS5 | 9726 | AG | A | Asp720fs | 0.80 |
| NS3 | 5026 | G | A | Glu169Lys | 0.61 | NS5 | 9902 | C | G | Ala778Gly | 3.40 |
| NS3 | 5304 | C | G | Cys261Trp | 0.98 | NS5 | 10045 | G | A | Asp826Asn | 1.97 |
| NS3 | 5433 | A | T | - | 1.58 | 3UTR | 10607 | C | CA | - | 0.53 |
| NS3 | 5436 | G | A | - | 1.59 | 3UTR | 10612 | C | CA | - | 1.15 |

| **179** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | | | | | | NS3 | 5478 | A | G | - | 1.47 |
| Primary | | | | | | NS3 | 5502 | A | G | - | 1.33 |
| 3.40E+04 | | | | | | NS3 | 5531 | G | T | Arg337Ile | 1.26 |
| 0 | | | | | | NS3 | 5543 | A | C | Glu341Ala | 0.87 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5615 | TA | T | Ala367fs | 0.53 |
| C | 135 | C | T | - | 1.67 | NS3 | 5730 | T | C | - | 1.12 |
| C | 341 | G | T | Arg82Ile | 1.82 | NS3 | 5839 | C | T | Arg440Trp | 2.68 |
| prM/M | 450 | C | T | - | 0.60 | NS3 | 5844 | A | G | - | 1.05 |
| prM/M | 606 | C | T | - | 0.78 | NS3 | 5915 | C | CA | Asn467fs | 6.60 |
| prM/M | 703 | G | GA | Arg91fs | 2.84 | NS3 | 5979 | C | A | - | 2.62 |
| prM/M | 834 | C | T | - | 0.69 | NS3 | 6094 | C | T | - | 1.94 |
| E | 1040 | C | CA | Asn37fs | 3.19 | NS3 | 6105 | G | A | - | 8.30 |
| E | 1124 | CA | C | Lys64fs | 0.84 | NS3 | 6157 | C | T | - | 2.80 |
| E | 1287 | T | C | - | 1.00 | NS3 | 6198 | T | C | - | 3.01 |
| E | 1302 | A | G | - | 1.19 | NS3 | 6285 | G | A | - | 1.40 |
| E | 1311 | G | A | Met125Ile | 1.24 | NS3 | 6285 | G | GA | Lys590fs | 2.46 |
| E | 1353 | T | C | - | 1.10 | NS3 | 6365 | C | T | Ala615Val | 0.83 |
| E | 1595 | G | T | Trp220Leu | 1.02 | NS4A | 6462 | C | T | - | 0.75 |
| E | 1695 | A | G | - | 1.14 | NS4A | 6496 | C | T | His41Tyr | 0.80 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1710 | T | G | - | 1.61 | NS4A | 6500 | A | C | Asn42Thr | 0.82 |
| E | 1963 | G | GA | Arg345fs | 0.53 | NS4A | 6528 | C | T | - | 0.83 |
| E | 1986 | C | T | - | 0.76 | NS4A | 6585 | A | G | - | 0.93 |
| E | 1994 | C | CGATTG | Val354fs | 0.75 | NS4A | 6681 | G | A | - | 1.06 |
| E | 2247 | C | T | - | 0.57 | NS4A | 6711 | G | GT | Leu115fs | 3.22 |
| E | 2269 | G | A | Gly445Arg | 0.60 | NS4A | 6783 | T | C | - | 1.30 |
| E | 2276 | T | C | Val447Ala | 1.65 | NS4A | 6790 | A | T | Ile139Leu | 1.86 |
| E | 2308 | C | T | Leu458Phe | 0.90 | NS4B | 6844 | G | A | Glu7Lys | 1.31 |
| E | 2315 | G | T | Gly460Val | 1.11 | NS4B | 6922 | C | T | Arg33Cys | 1.00 |
| E | 2319 | C | A | - | 0.94 | NS4B | 6982 | C | T | Arg53* | 1.24 |
| E | 2397 | G | A | - | 0.58 | NS4B | 7038 | G | A | - | 1.03 |
| NS1 | 2448 | A | G | - | 1.21 | NS4B | 7049 | T | C | Leu75Ser | 0.79 |
| NS1 | 2484 | T | C | - | 1.08 | NS4B | 7137 | T | C | - | 0.86 |
| NS1 | 2991 | C | T | - | 0.70 | NS4B | 7435 | C | G | Leu204Val | 0.84 |
| NS1 | 3027 | G | A | Met202Ile | 2.18 | NS4B | 7441 | G | A | Glu206Lys | 0.73 |
| NS1 | 3162 | A | T | Leu247Phe | 1.77 | NS4B | 7457 | G | A | Arg211Lys | 1.12 |
| NS1 | 3166 | G | T | Gly249* | 1.81 | NS5 | 7645 | C | A | Gln26Lys | 1.37 |
| NS1 | 3241 | G | GCCT | Glu274delinsAlaTer | 1.94 | NS5 | 7656 | G | A | - | 3.01 |
| NS1 | 3330 | C | T | - | 0.62 | NS5 | 7720 | G | A | Asp51Asn | 1.70 |
| NS1 | 3402 | C | T | - | 0.78 | NS5 | 7758 | G | A | - | 2.07 |
| NS1 | 3438 | G | A | - | 0.53 | NS5 | 7920 | A | C | - | 1.13 |
| NS2A | 3522 | G | T | Met15Ile | 0.61 | NS5 | 7999 | C | T | - | 0.54 |
| NS2A | 3716 | C | T | Ala80Val | 0.61 | NS5 | 8074 | G | C | Glu169Gln | 2.12 |
| NS2A | 3801 | T | C | - | 1.37 | NS5 | 8143 | G | GA | Met194fs | 0.58 |
| NS2A | 3866 | G | C | Gly130Ala | 1.04 | NS5 | 8184 | G | C | Leu205Phe | 0.55 |
| NS2A | 3910 | C | T | - | 1.17 | NS5 | 8298 | C | T | - | 0.61 |
| NS2A | 3928 | G | C | Ala151Pro | 1.01 | NS5 | 8544 | T | C | - | 1.22 |
| NS2B | 4206 | T | C | - | 0.98 | NS5 | 8569 | A | G | Ile334Val | 1.61 |
| NS2B | 4249 | A | G | Ile40Val | 0.98 | NS5 | 8891 | T | TA | His442fs | 0.58 |
| NS2B | 4257 | T | C | - | 1.14 | NS5 | 8926 | A | AC | Asn453fs | 2.91 |
| NS2B | 4341 | T | C | - | 1.34 | NS5 | 8932 | A | T | Met455Leu | 1.91 |
| NS2B | 4482 | G | A | - | 0.82 | NS5 | 9705 | T | TC | Phe713fs | 1.38 |
| NS3 | 4938 | C | T | - | 1.19 | NS5 | 9726 | AG | A | Asp720fs | 0.57 |
| NS3 | 4959 | C | T | - | 0.61 | NS5 | 9795 | G | A | - | 0.99 |
| NS3 | 5168 | T | G | Leu216Trp | 1.35 | NS5 | 9902 | C | G | Ala778Gly | 2.48 |
| NS3 | 5304 | T | C | - | 1.63 | NS5 | 9942 | C | T | - | 1.25 |
| NS3 | 5365 | A | G | Ile282Val | 0.96 | 3UTR | 10389 | C | T | - | 0.67 |
| NS3 | 5436 | G | A | - | 1.55 | 3UTR | 10608 | C | CA | - | 0.88 |

| **180** | | | | | |
|---|---|---|---|---|---|
| DF | | | | | |
| Primary | | | | | |
| 3.08E+04 | | | | | |
| 2 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 110 | G | GA | Lys6fs | 0.71 |
| C | 135 | C | T | - | 1.54 |
| C | 341 | G | T | Arg82Ile | 1.56 |
| prM/M | 549 | G | A | Met37Ile | 1.76 |
| prM/M | 703 | G | GA | Arg91fs | 2.34 |
| prM/M | 834 | C | T | - | 1.23 |
| E | 1102 | T | C | - | 1.00 |
| E | 1313 | A | C | Glu126Ala | 1.36 |
| E | 1595 | G | T | Trp220Leu | 1.59 |
| E | 1935 | T | C | - | 11.42 |
| E | 1963 | G | A | Glu343Lys | 0.68 |
| E | 1994 | C | CGATTG | Val354fs | 1.10 |
| E | 2210 | T | A | Leu425* | 1.11 |
| E | 2303 | A | G | Lys456Arg | 1.36 |
| NS3 | 5309 | C | A | Ala263Asp | 1.35 |
| NS3 | 5436 | G | A | - | 0.54 |
| NS3 | 5531 | G | T | Arg337Ile | 0.69 |
| NS3 | 5642 | G | T | Cys374Phe | 0.59 |
| NS3 | 5717 | C | T | Ala399Val | 0.84 |
| NS3 | 5839 | C | T | Arg440Trp | 1.34 |
| NS3 | 5909 | G | GA | Asn464fs | 0.57 |
| NS3 | 6105 | G | A | - | 7.57 |
| NS3 | 6285 | G | GA | Lys590fs | 1.33 |
| NS3 | 6365 | C | T | Ala615Val | 0.68 |
| NS4A | 6790 | A | T | Ile139Leu | 1.50 |
| NS4B | 6828 | C | T | - | 4.28 |
| NS4B | 6844 | G | A | Glu7Lys | 1.25 |
| NS4B | 6922 | C | T | Arg33Cys | 0.82 |
| NS4B | 6958 | A | G | Thr45Ala | 0.66 |
| NS4B | 6982 | C | T | Arg53* | 1.40 |
| NS4B | 7011 | T | C | - | 1.34 |
| NS4B | 7152 | A | T | - | 1.02 |
| NS5 | 7763 | T | C | Phe65Ser | 5.33 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 2308 | C | T | Leu458Phe | 1.48 | NS5 | 8074 | G | C | Glu169Gln | 1.57 |
| E | 2315 | G | T | Gly460Val | 1.09 | NS5 | 8143 | G | GA | Met194fs | 1.24 |
| NS1 | 2649 | T | C | - | 6.55 | NS5 | 8602 | G | A | Asp345Asn | 1.47 |
| NS1 | 3152 | C | CA | Asn246fs | 3.28 | NS5 | 8871 | G | A | - | 2.28 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 1.37 | NS5 | 8891 | T | TA | His442fs | 1.63 |
| NS1 | 3398 | A | G | Glu326Gly | 1.04 | NS5 | 8926 | A | AC | Asn453fs | 1.38 |
| NS2A | 3672 | G | T | - | 0.85 | NS5 | 9043 | C | T | - | 0.92 |
| NS2A | 3720 | C | CA | Val83fs | 1.14 | NS5 | 9705 | T | TC | Phe713fs | 1.18 |
| NS2A | 3866 | G | C | Gly130Ala | 1.02 | NS5 | 9714 | G | C | Glu715Asp | 0.78 |
| NS2A | 3928 | G | C | Ala151Pro | 1.08 | NS5 | 9716 | T | A | Leu716* | 0.79 |
| NS3 | 4878 | T | C | - | 1.18 | NS5 | 9718 | G | C | Val717Leu | 0.81 |
| NS3 | 4887 | C | T | - | 0.56 | NS5 | 9726 | AG | A | Asp720fs | 0.89 |
| NS3 | 4941 | C | T | - | 0.79 | NS5 | 9902 | C | G | Ala778Gly | 3.08 |
| NS3 | 5208 | T | C | - | 1.28 | NS5 | 10050 | G | A | - | 16.15 |
| NS3 | 5304 | T | G | Cys261Trp | 1.53 | 3UTR | 10613 | C | CA | - | 0.71 |

| **181** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DF | | | | | | NS3 | 5283 | G | A | - | 7.26 |
| Secondary | | | | | | NS3 | 5304 | C | T | - | 6.26 |
| 7.84E+04 | | | | | | NS3 | 5340 | C | T | - | 1.74 |
| 2 | | | | | | NS3 | 5451 | A | C | - | 2.65 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5460 | C | T | - | 3.52 |
| C | 111 | A | G | - | 3.59 | NS3 | 5508 | T | C | - | 3.70 |
| C | 135 | C | T | - | 1.82 | NS3 | 5531 | G | T | Arg337Ile | 4.23 |
| C | 201 | C | T | - | 8.64 | NS3 | 5532 | A | G | - | 3.51 |
| C | 219 | C | T | - | 1.03 | NS3 | 5544 | G | A | - | 4.27 |
| C | 219 | C | A | - | 1.20 | NS3 | 5550 | A | G | - | 4.27 |
| C | 228 | A | G | - | 1.12 | NS3 | 5559 | A | T | - | 5.21 |
| C | 312 | C | CA | Ser75fs | 1.92 | NS3 | 5574 | C | T | - | 5.94 |
| C | 315 | A | G | - | 8.54 | NS3 | 5583 | T | C | - | 4.98 |
| C | 336 | C | T | - | 6.87 | NS3 | 5610 | A | G | - | 4.45 |
| C | 345 | G | C | - | 1.31 | NS3 | 5613 | C | T | - | 9.77 |
| C | 348 | C | T | - | 1.32 | NS3 | 5631 | C | T | - | 4.23 |
| C | 375 | C | T | - | 1.91 | NS3 | 5672 | A | AT | Gln384fs | 1.04 |
| C | 384 | T | C | - | 4.11 | NS3 | 5688 | T | C | - | 3.92 |
| C | 387 | A | G | - | 5.82 | NS3 | 5694 | C | T | - | 2.39 |
| C | 431 | C | T | Ala112Val | 5.85 | NS3 | 5700 | G | A | - | 2.15 |
| prM/M | 445 | C | T | - | 8.06 | NS3 | 5704 | G | A | Val395Ile | 1.78 |
| prM/M | 508 | T | C | - | 8.67 | NS3 | 5715 | A | G | - | 2.23 |
| prM/M | 523 | G | A | Asp29Asn | 5.40 | NS3 | 5716 | G | A | Ala399Thr | 2.17 |
| prM/M | 525 | T | C | - | 5.66 | NS3 | 5742 | A | G | - | 2.55 |
| prM/M | 531 | T | C | - | 6.76 | NS3 | 5766 | C | T | - | 1.94 |
| prM/M | 567 | A | G | - | 7.17 | NS3 | 5781 | G | A | - | 1.97 |
| prM/M | 648 | T | C | - | 46.47 | NS3 | 5796 | T | C | - | 1.61 |
| prM/M | 651 | A | G | - | 8.53 | NS3 | 5799 | A | G | - | 1.61 |
| prM/M | 717 | G | A | - | 19.30 | NS3 | 5802 | T | C | - | 9.27 |
| prM/M | 792 | A | G | - | 8.01 | NS3 | 5817 | T | C | - | 2.35 |
| prM/M | 798 | C | T | - | 7.97 | NS3 | 5829 | T | C | - | 1.77 |
| prM/M | 834 | C | T | - | 7.71 | NS3 | 5832 | C | T | - | 1.74 |
| prM/M | 837 | C | T | - | 8.01 | NS3 | 5835 | G | A | - | 8.63 |
| prM/M | 852 | A | G | - | 9.50 | NS3 | 5847 | T | C | - | 6.84 |
| prM/M | 855 | C | T | - | 0.93 | NS3 | 5859 | T | C | - | 4.46 |
| prM/M | 856 | T | C | - | 9.27 | NS3 | 5868 | A | G | - | 7.11 |
| prM/M | 864 | T | C | - | 10.24 | NS3 | 5915 | C | CA | Asn467fs | 15.12 |
| prM/M | 873 | C | A | - | 13.29 | NS3 | 6009 | C | T | - | 5.41 |
| prM/M | 876 | G | A | - | 1.35 | NS3 | 6033 | C | T | - | 7.12 |
| prM/M | 880 | C | T | His148Tyr | 1.33 | NS3 | 6048 | C | T | - | 1.90 |
| prM/M | 882 | C | T | - | 1.29 | NS3 | 6069 | T | G | - | 11.73 |
| prM/M | 891 | G | A | - | 1.43 | NS3 | 6094 | C | T | - | 12.24 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| prM/M | 894 | A | C | - | 17.04 | NS3 | 6099 | G | A | - | 9.94 |
| prM/M | 912 | A | G | - | 23.21 | NS3 | 6108 | A | G | - | 2.68 |
| prM/M | 921 | C | T | - | 1.96 | NS3 | 6157 | T | C | - | 10.43 |
| E | 954 | C | A | - | 21.36 | NS3 | 6159 | G | A | - | 7.69 |
| E | 960 | T | C | - | 3.99 | NS3 | 6192 | C | T | - | 1.32 |
| E | 969 | C | T | - | 3.46 | NS3 | 6203 | A | G | Lys588Arg | 2.11 |
| E | 972 | A | G | - | 3.17 | NS3 | 6220 | G | A | Val567Ile | 5.34 |
| E | 1005 | A | C | - | 16.67 | NS3 | 6222 | C | T | - | 5.69 |
| E | 1008 | T | C | - | 3.66 | NS3 | 6237 | A | C | - | 3.17 |
| E | 1023 | T | C | - | 17.08 | NS3 | 6238 | C | T | - | 3.02 |
| E | 1032 | G | A | - | 1.68 | NS3 | 6279 | G | A | - | 4.55 |
| E | 1040 | C | CA | Asn37fs | 4.40 | NS3 | 6285 | G | A | - | 2.98 |
| E | 1062 | T | C | - | 2.07 | NS3 | 6285 | G | GA | Lys590fs | 1.73 |
| E | 1068 | A | G | - | 2.08 | NS3 | 6288 | A | G | - | 4.80 |
| E | 1089 | A | G | - | 2.33 | NS3 | 6294 | A | G | - | 4.22 |
| E | 1102 | T | C | - | 14.55 | NS3 | 6300 | T | C | - | 14.29 |
| E | 1122 | A | G | - | 3.33 | NS3 | 6303 | A | G | - | 3.20 |
| E | 1266 | A | G | - | 47.32 | NS3 | 6307 | T | C | - | 3.16 |
| E | 1287 | T | C | - | 1.76 | NS3 | 6312 | T | C | - | 3.97 |
| E | 1293 | C | T | - | 2.46 | NS3 | 6333 | A | G | - | 2.87 |
| E | 1396 | G | A | Asp154Asn | 1.79 | NS3 | 6339 | A | G | - | 2.56 |
| E | 1413 | C | A | - | 2.75 | NS3 | 6342 | A | C | - | 2.49 |
| E | 1473 | T | C | - | 3.05 | NS3 | 6345 | G | A | - | 2.91 |
| E | 1545 | C | T | - | 6.94 | NS3 | 6357 | A | G | - | 2.91 |
| E | 1590 | A | G | - | 9.66 | NS3 | 6366 | T | C | - | 3.33 |
| E | 1595 | G | T | Trp220Leu | 2.18 | NS4A | 6378 | C | T | - | 7.83 |
| E | 1599 | A | G | - | 8.04 | NS4A | 6379 | C | T | - | 7.83 |
| E | 1626 | T | C | - | 9.29 | NS4A | 6423 | C | T | - | 2.81 |
| E | 1641 | A | G | - | 9.77 | NS4A | 6429 | T | C | - | 2.09 |
| E | 1858 | A | G | Ile308Val | 4.81 | NS4A | 6435 | G | A | - | 1.98 |
| E | 1860 | T | C | - | 14.06 | NS4A | 6441 | A | G | - | 1.98 |
| E | 1863 | A | G | - | 12.89 | NS4A | 6444 | C | T | - | 2.05 |
| E | 1944 | T | C | - | 4.97 | NS4A | 6448 | C | T | - | 2.04 |
| E | 1959 | T | C | - | 5.70 | NS4A | 6462 | C | T | - | 11.95 |
| E | 1972 | C | T | His346Tyr | 4.71 | NS4A | 6491 | G | A | Arg39Lys | 12.79 |
| E | 1995 | G | A | - | 5.89 | NS4A | 6496 | C | T | His41Tyr | 13.72 |
| E | 2023 | G | A | Ala363Ser | 4.73 | NS4A | 6498 | C | T | - | 6.40 |
| E | 2024 | C | G | | 4.70 | NS4A | 6500 | A | C | Asn42Thr | 11.89 |
| E | 2049 | T | C | - | 1.74 | NS4A | 6512 | G | A | Ser46Asn | 12.35 |
| E | 2055 | C | T | - | 0.95 | NS4A | 6528 | C | T | - | 13.27 |
| E | 2067 | T | C | - | 2.16 | NS4A | 6544 | C | T | - | 1.12 |
| E | 2073 | T | C | - | 3.01 | NS4A | 6547 | T | C | - | 11.76 |
| E | 2223 | C | T | - | 6.59 | NS4A | 6556 | T | C | - | 9.22 |
| E | 2247 | C | T | - | 6.96 | NS4A | 6562 | A | G | Thr63Ala | 8.82 |
| E | 2256 | C | T | - | 1.62 | NS4A | 6582 | C | T | - | 1.61 |
| E | 2289 | T | C | - | 8.40 | NS4A | 6585 | A | G | - | 1.67 |
| E | 2307 | T | C | - | 8.21 | NS4A | 6588 | C | T | - | 1.60 |
| E | 2319 | A | C | - | 6.92 | NS4A | 6591 | A | G | - | 1.86 |
| E | 2376 | A | G | - | 5.82 | NS4A | 6615 | G | A | - | 1.58 |
| NS1 | 2434 | G | A | Val5Ile | 7.00 | NS4A | 6633 | C | T | - | 7.80 |
| NS1 | 2448 | G | A | - | 8.21 | NS4A | 6654 | C | T | - | 8.79 |
| NS1 | 2451 | C | T | - | 6.70 | NS4A | 6681 | G | A | - | 8.86 |
| NS1 | 2484 | C | T | - | 6.77 | NS4A | 6711 | G | GT | Leu115fs | 2.64 |
| NS1 | 2511 | A | G | - | 7.92 | NS4A | 6732 | C | T | - | 2.86 |
| NS1 | 2589 | G | A | - | 5.37 | NS4A | 6777 | G | A | - | 4.03 |
| NS1 | 2673 | G | A | - | 2.86 | NS4A | 6780 | C | T | - | 3.75 |
| NS1 | 2781 | A | G | - | 4.97 | NS4B | 6833 | T | TG | Leu6fs | 0.65 |
| NS1 | 2802 | GTCT | G | Ser128del | 0.73 | NS4B | 6837 | T | G | - | 3.25 |
| NS1 | 2835 | T | C | - | 2.03 | NS4B | 6840 | C | T | - | 3.37 |
| NS1 | 2878 | A | C | Met153Leu | 5.28 | NS4B | 6841 | T | C | - | 16.83 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS1 | 2898 | C | T | - | 7.89 | NS4B | 6844 | G | GA | Thr9fs | 1.24 |
| NS1 | 2961 | T | C | - | 10.69 | NS4B | 6852 | C | G | - | 3.58 |
| NS1 | 2976 | G | A | - | 9.40 | NS4B | 6861 | C | T | - | 3.26 |
| NS1 | 2989 | G | A | Asp190Asn | 7.78 | NS4B | 6862 | C | T | Leu13Phe | 3.25 |
| NS1 | 2991 | C | T | - | 1.04 | NS4B | 6864 | C | T | - | 1.74 |
| NS1 | 3009 | C | T | - | 6.46 | NS4B | 6870 | T | G | Phe15Leu | 3.05 |
| NS1 | 3033 | T | C | - | 1.44 | NS4B | 6871 | G | A | Gly16Arg | 2.43 |
| NS1 | 3036 | A | G | - | 1.44 | NS4B | 6879 | C | T | - | 12.60 |
| NS1 | 3075 | C | T | - | 10.09 | NS4B | 6922 | C | T | Arg33Cys | 1.09 |
| NS1 | 3081 | T | A | - | 6.08 | NS4B | 6924 | T | C | - | 2.36 |
| NS1 | 3102 | G | A | - | 2.20 | NS4B | 6942 | G | C | - | 2.07 |
| NS1 | 3111 | C | T | - | 2.02 | NS4B | 6948 | C | T | - | 10.66 |
| NS1 | 3120 | C | T | - | 1.71 | NS4B | 6957 | T | C | - | 10.21 |
| NS1 | 3129 | A | G | - | 2.65 | NS4B | 6967 | G | A | Val48Ile | 2.26 |
| NS1 | 3152 | C | CA | Asn246fs | 5.05 | NS4B | 6979 | T | C | - | 1.83 |
| NS1 | 3165 | C | T | - | 2.56 | NS4B | 6982 | C | T | Arg53* | 1.13 |
| NS1 | 3168 | G | A | - | 2.37 | NS4B | 6982 | C | A | - | 1.83 |
| NS1 | 3234 | C | T | - | 7.56 | NS4B | 6990 | C | T | - | 1.92 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 0.88 | NS4B | 7008 | G | A | - | 7.86 |
| NS1 | 3330 | T | C | - | 7.94 | NS4B | 7014 | C | G | - | 1.61 |
| NS1 | 3398 | A | G | Glu326Gly | 2.29 | NS4B | 7017 | C | T | - | 1.86 |
| NS1 | 3402 | C | T | - | 8.28 | NS4B | 7018 | C | T | - | 7.74 |
| NS1 | 3432 | C | T | - | 7.99 | NS4B | 7032 | T | C | - | 2.05 |
| NS1 | 3438 | G | A | - | 8.35 | NS4B | 7041 | C | T | - | 8.07 |
| NS1 | 3447 | G | A | - | 8.52 | NS4B | 7059 | C | T | - | 10.25 |
| NS1 | 3474 | G | A | - | 4.19 | NS4B | 7062 | A | G | - | 8.79 |
| NS2A | 3597 | T | C | - | 2.31 | NS4B | 7083 | A | G | - | 8.85 |
| NS2A | 3624 | C | T | - | 5.73 | NS4B | 7089 | C | T | - | 1.65 |
| NS2A | 3629 | A | G | Lys51Arg | 7.08 | NS4B | 7092 | C | T | - | 1.75 |
| NS2A | 3663 | T | C | - | 10.20 | NS4B | 7101 | C | T | - | 1.72 |
| NS2A | 3720 | C | CA | Val83fs | 0.71 | NS4B | 7104 | C | T | - | 1.66 |
| NS2A | 3753 | T | C | - | 9.07 | NS4B | 7107 | T | C | - | 1.96 |
| NS2A | 3762 | A | G | - | 1.17 | NS4B | 7110 | C | T | - | 1.94 |
| NS2A | 3768 | T | C | - | 9.93 | NS4B | 7116 | A | G | - | 2.49 |
| NS2A | 3787 | G | A | Ala104Thr | 0.90 | NS4B | 7122 | C | T | - | 2.42 |
| NS2A | 3858 | A | G | - | 2.15 | NS4B | 7137 | C | T | - | 7.84 |
| NS2A | 3864 | A | G | - | 8.15 | NS4B | 7143 | C | T | - | 1.61 |
| NS2A | 3866 | G | C | Gly130Ala | 0.87 | NS4B | 7146 | C | T | - | 1.89 |
| NS2A | 3874 | G | A | Val133Ile | 1.69 | NS4B | 7155 | T | C | - | 2.08 |
| NS2A | 3875 | T | C | Val133Ala | 9.21 | NS4B | 7158 | T | G | - | 1.32 |
| NS2A | 3876 | C | T | - | 2.11 | NS4B | 7165 | C | T | - | 12.25 |
| NS2A | 3879 | A | C | - | 10.54 | NS4B | 7182 | C | T | - | 2.32 |
| NS2A | 3906 | C | T | - | 1.36 | NS4B | 7185 | T | C | - | 2.97 |
| NS2A | 3912 | G | A | - | 1.71 | NS4B | 7186 | A | G | ile121Val | 10.49 |
| NS2A | 3921 | C | T | - | 10.42 | NS4B | 7248 | T | C | - | 16.38 |
| NS2A | 3928 | G | C | Ala151Pro | 0.99 | NS4B | 7314 | G | A | - | 7.87 |
| NS2A | 3935 | C | T | Ser153Leu | 1.70 | NS4B | 7356 | T | C | - | 5.36 |
| NS2A | 3936 | G | A | - | 1.51 | NS4B | 7383 | C | T | - | 5.75 |
| NS2A | 3942 | C | T | - | 1.68 | NS4B | 7410 | C | T | - | 3.89 |
| NS2A | 3958 | T | C | - | 1.46 | NS4B | 7428 | T | C | - | 4.05 |
| NS2A | 3960 | G | A | - | 1.54 | NS5 | 7656 | A | G | - | 5.31 |
| NS2A | 3966 | C | T | - | 1.49 | NS5 | 7689 | A | G | - | 2.02 |
| NS2A | 3975 | G | A | - | 1.44 | NS5 | 7719 | G | A | - | 3.92 |
| NS2A | 3991 | T | C | - | 1.39 | NS5 | 7776 | T | C | - | 5.44 |
| NS2A | 3998 | C | T | Ala174Val | 1.75 | NS5 | 7794 | G | A | - | 3.88 |
| NS2A | 4009 | T | A | Ser178Thr | 1.51 | NS5 | 7812 | C | T | - | 8.40 |
| NS2A | 4011 | C | T | - | 6.71 | NS5 | 7911 | T | C | - | 7.68 |
| NS2A | 4021 | C | T | - | 1.26 | NS5 | 7947 | G | T | - | 1.35 |
| NS2A | 4034 | G | A | Arg186Gln | 8.43 | NS5 | 7956 | G | A | - | 1.44 |
| NS2A | 4047 | T | C | - | 2.28 | NS5 | 7959 | T | C | - | 8.08 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2A | 4057 | C | T | - | 2.54 | NS5 | 7971 | C | T | - | 1.18 |
| NS2A | 4069 | G | A | Val198Ile | 13.65 | NS5 | 7996 | C | T | - | 8.20 |
| NS2A | 4071 | A | C | - | 2.48 | NS5 | 8035 | A | G | Ile156Val | 1.24 |
| NS2A | 4074 | A | G | - | 2.88 | NS5 | 8049 | G | A | - | 6.77 |
| NS2A | 4083 | T | C | - | 9.13 | NS5 | 8070 | G | A | - | 9.27 |
| NS2A | 4098 | T | C | - | 2.88 | NS5 | 8074 | G | C | Glu169Gln | 2.71 |
| NS2A | 4107 | T | C | - | 2.85 | NS5 | 8083 | T | C | - | 8.54 |
| NS2A | 4110 | C | T | - | 11.48 | NS5 | 8121 | C | T | - | 6.74 |
| NS2A | 4113 | G | A | - | 2.46 | NS5 | 8143 | G | GA | Met194fs | 1.78 |
| NS2A | 4121 | G | A | Ser215Asn | 3.17 | NS5 | 8184 | G | A | - | 6.39 |
| NS2B | 4134 | C | T | - | 2.79 | NS5 | 8214 | G | A | - | 1.13 |
| NS2B | 4140 | G | A | - | 2.76 | NS5 | 8235 | T | C | - | 3.16 |
| NS2B | 4149 | A | G | - | 2.46 | NS5 | 8241 | C | T | - | 14.53 |
| NS2B | 4164 | T | C | - | 8.55 | NS5 | 8242 | A | T | Thr225Ser | 2.94 |
| NS2B | 4197 | A | G | - | 2.56 | NS5 | 8284 | C | T | - | 12.54 |
| NS2B | 4206 | C | T | - | 6.29 | NS5 | 8292 | C | T | - | 31.33 |
| NS2B | 4212 | T | C | - | 2.14 | NS5 | 8298 | T | C | - | 13.16 |
| NS2B | 4221 | T | A | - | 1.85 | NS5 | 8310 | C | T | - | 6.31 |
| NS2B | 4225 | T | C | - | 1.73 | NS5 | 8508 | A | G | - | 4.45 |
| NS2B | 4242 | C | T | - | 1.83 | NS5 | 8526 | T | C | - | 3.15 |
| NS2B | 4248 | C | T | - | 1.71 | NS5 | 8535 | C | T | - | 2.79 |
| NS2B | 4251 | A | G | - | 1.94 | NS5 | 8541 | G | A | - | 1.83 |
| NS2B | 4270 | C | A | - | 1.75 | NS5 | 8544 | T | C | - | 2.82 |
| NS2B | 4278 | T | C | - | 7.33 | NS5 | 8572 | G | A | Val335Ile | 1.88 |
| NS2B | 4305 | T | C | - | 1.95 | NS5 | 8577 | T | C | - | 2.01 |
| NS2B | 4329 | G | A | - | 6.19 | NS5 | 8619 | G | A | - | 3.52 |
| NS2B | 4338 | G | A | - | 6.71 | NS5 | 8640 | GA | G | Val359fs | 1.53 |
| NS2B | 4344 | T | C | - | 6.06 | NS5 | 8745 | G | A | - | 14.29 |
| NS2B | 4350 | C | T | - | 6.50 | NS5 | 8751 | T | C | - | 9.09 |
| NS2B | 4414 | T | C | - | 9.52 | NS5 | 8803 | A | G | Ile412Val | 3.63 |
| NS2B | 4443 | G | T | - | 6.47 | NS5 | 8859 | G | T | Arg430Ser | 4.47 |
| NS2B | 4482 | A | G | - | 7.33 | NS5 | 8926 | A | AC | Asn453fs | 2.65 |
| NS2B | 4491 | G | A | - | 5.78 | NS5 | 8967 | T | C | - | 5.07 |
| NS2B | 4509 | T | G | - | 8.03 | NS5 | 9006 | T | C | - | 1.70 |
| NS3 | 4557 | A | C | - | 2.47 | NS5 | 9012 | A | G | - | 4.57 |
| NS3 | 4566 | A | G | - | 4.73 | NS5 | 9018 | C | T | - | 1.52 |
| NS3 | 4573 | C | T | - | 5.08 | NS5 | 9066 | T | C | - | 7.79 |
| NS3 | 4587 | C | T | - | 1.98 | NS5 | 9079 | T | C | - | 9.61 |
| NS3 | 4596 | T | C | - | 9.77 | NS5 | 9112 | T | C | - | 4.76 |
| NS3 | 4602 | A | G | - | 4.94 | NS5 | 9120 | C | T | - | 5.31 |
| NS3 | 4604 | G | A | Arg28Lys | 3.92 | NS5 | 9345 | C | T | - | 12.20 |
| NS3 | 4620 | T | C | - | 3.52 | NS5 | 9356 | A | G | Lys596Arg | 10.87 |
| NS3 | 4623 | T | C | - | 3.57 | NS5 | 9375 | G | A | - | 6.78 |
| NS3 | 4629 | T | C | - | 4.87 | NS5 | 9384 | C | T | - | 7.14 |
| NS3 | 4635 | C | T | - | 36.11 | NS5 | 9399 | C | T | - | 3.17 |
| NS3 | 4641 | T | C | - | 5.16 | NS5 | 9424 | C | T | - | 2.78 |
| NS3 | 4662 | T | C | - | 3.45 | NS5 | 9477 | T | C | - | 2.93 |
| NS3 | 4695 | G | A | - | 2.33 | NS5 | 9501 | C | G | His644Gln | 3.58 |
| NS3 | 4703 | G | A | Arg61Lys | 2.21 | NS5 | 9576 | C | T | - | 9.91 |
| NS3 | 4707 | A | G | - | 5.36 | NS5 | 9606 | A | G | - | 1.63 |
| NS3 | 4731 | A | G | - | 2.25 | NS5 | 9705 | T | TC | Phe713fs | 1.05 |
| NS3 | 4739 | A | G | Lys73Arg | 2.29 | NS5 | 9714 | G | C | Glu715Asp | 1.01 |
| NS3 | 4746 | C | T | - | 4.22 | NS5 | 9726 | AG | A | Asp720fs | 1.25 |
| NS3 | 4755 | A | G | - | 2.05 | NS5 | 9745 | G | A | Val726Ile | 31.26 |
| NS3 | 4812 | T | C | - | 4.47 | NS5 | 9795 | A | G | - | 10.22 |
| NS3 | 4830 | G | A | - | 6.22 | NS5 | 9825 | C | T | - | 9.72 |
| NS3 | 4905 | A | G | - | 7.12 | NS5 | 9888 | A | G | - | 5.55 |
| NS3 | 4944 | T | C | - | 6.52 | NS5 | 9942 | T | C | - | 2.36 |
| NS3 | 4959 | C | T | - | 5.54 | NS5 | 10047 | C | CA | Thr828fs | 0.55 |
| NS3 | 4971 | T | C | - | 4.20 | NS5 | 10075 | G | A | Val836Ile | 8.94 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS3 | 4974 | C | T | - | 5.40 | NS5 | 10083 | T | C | - | 9.99 |
| NS3 | 5004 | T | C | - | 3.77 | NS5 | 10155 | C | T | - | 7.31 |
| NS3 | 5026 | G | GA | Ser171fs | 3.31 | NS5 | 10202 | A | G | Glu878Gly | 7.01 |
| NS3 | 5070 | T | C | - | 7.32 | NS5 | 10252 | A | G | Lys895Glu | 6.80 |
| NS3 | 5082 | A | G | - | 5.87 | 3UTR | 10389 | CA | C | - | 17.46 |
| NS3 | 5190 | T | C | - | 7.89 | 3UTR | 10389 | C | CA | - | 0.68 |
| NS3 | 5270 | A | G | Glu250Gly | 1.87 | 3UTR | 10408 | C | T | - | 18.33 |
| NS3 | 5277 | T | C | - | 6.38 | 3UTR | 10612 | C | CA | - | 4.30 |

| **182** | | | | | |
|---|---|---|---|---|---|
| DF | | | | | |
| Primary | | | | | |
| 4.23E+04 | | | | | |
| 2 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 135 | C | T | - | 2.55 |
| C | 201 | C | T | - | 4.74 |
| C | 315 | A | G | - | 6.13 |
| C | 327 | T | C | - | 6.44 |
| C | 336 | C | T | - | 8.80 |
| C | 384 | T | C | - | 2.97 |
| C | 387 | A | G | - | 3.45 |
| prM/M | 445 | C | T | - | 3.73 |
| prM/M | 450 | T | C | - | 4.58 |
| prM/M | 457 | A | C | Asn7His | 2.79 |
| prM/M | 508 | T | C | - | 4.13 |
| prM/M | 567 | A | G | - | 3.15 |
| prM/M | 606 | C | T | - | 8.26 |
| prM/M | 651 | A | G | - | 7.02 |
| prM/M | 717 | G | A | - | 7.58 |
| prM/M | 834 | C | T | - | 3.25 |
| prM/M | 837 | C | T | - | 2.77 |
| prM/M | 852 | A | G | - | 3.38 |
| prM/M | 856 | T | C | - | 3.61 |
| prM/M | 864 | T | C | - | 3.68 |
| prM/M | 873 | C | A | - | 3.38 |
| prM/M | 894 | A | C | - | 3.86 |
| prM/M | 912 | A | G | - | 14.17 |
| prM/M | 921 | C | T | - | 4.29 |
| E | 954 | C | A | - | 6.48 |
| E | 1005 | A | C | - | 11.43 |
| E | 1040 | C | CA | Asn37fs | 4.62 |
| E | 1094 | C | T | Pro53Leu | 10.17 |
| E | 1102 | T | C | - | 13.79 |
| E | 1214 | A | G | Lys93Arg | 8.79 |
| E | 1311 | G | A | Met125Ile | 1.85 |
| E | 1415 | A | G | Lys160Arg | 4.55 |
| E | 1473 | T | C | - | 5.13 |
| E | 1590 | A | G | - | 7.10 |
| E | 1641 | A | G | - | 8.78 |
| E | 1689 | T | C | - | 2.81 |
| E | 1842 | T | C | - | 4.20 |
| E | 1860 | T | C | - | 18.87 |
| E | 1863 | A | G | - | 7.96 |
| E | 1911 | A | G | - | 4.84 |
| E | 1994 | C | CGATTG | Val354fs | 1.44 |
| E | 2023 | G | A | Ala363Ser | 2.86 |
| E | 2024 | C | G | | 3.24 |
| E | 2220 | G | A | - | 3.09 |
| NS3 | 4959 | C | T | - | 3.48 |
| NS3 | 4974 | C | T | - | 5.86 |
| NS3 | 5004 | T | C | - | 1.86 |
| NS3 | 5112 | A | G | - | 4.48 |
| NS3 | 5190 | T | C | - | 1.99 |
| NS3 | 5277 | T | C | - | 4.22 |
| NS3 | 5283 | G | A | - | 6.21 |
| NS3 | 5304 | C | T | - | 4.90 |
| NS3 | 5457 | T | TTCTCTTTCTTCA | Ile312Phe313insSerLeuSerSer | 2.22 |
| NS3 | 5526 | G | A | - | 5.93 |
| NS3 | 5613 | C | T | - | 5.60 |
| NS3 | 5649 | G | A | - | 4.08 |
| NS3 | 5667 | G | A | - | 6.67 |
| NS3 | 5835 | G | A | - | 6.78 |
| NS3 | 5847 | T | C | - | 5.71 |
| NS3 | 6069 | T | G | - | 5.93 |
| NS3 | 6094 | C | T | - | 6.29 |
| NS3 | 6099 | G | A | - | 5.75 |
| NS3 | 6222 | C | T | - | 4.64 |
| NS3 | 6300 | T | C | - | 5.30 |
| NS3 | 6330 | C | T | - | 3.61 |
| NS3 | 6365 | C | T | Ala615Val | 1.42 |
| NS4A | 6378 | C | T | - | 4.43 |
| NS4A | 6379 | C | T | - | 4.48 |
| NS4A | 6462 | C | T | - | 7.92 |
| NS4A | 6471 | T | C | - | 5.29 |
| NS4A | 6491 | G | A | Arg39Lys | 6.76 |
| NS4A | 6496 | C | T | His41Tyr | 9.76 |
| NS4A | 6500 | A | C | Asn42Thr | 7.97 |
| NS4A | 6528 | C | T | - | 11.20 |
| NS4A | 6547 | T | C | - | 14.29 |
| NS4A | 6556 | T | C | - | 11.79 |
| NS4A | 6562 | A | G | Thr63Ala | 10.16 |
| NS4A | 6585 | A | G | - | 7.02 |
| NS4A | 6633 | C | T | - | 5.88 |
| NS4A | 6654 | C | T | - | 8.76 |
| NS4A | 6681 | G | A | - | 9.68 |
| NS4A | 6711 | G | GT | Leu115fs | 2.13 |
| NS4A | 6724 | GT | G | Leu118fs | 2.07 |
| NS4B | 6841 | T | C | - | 4.96 |
| NS4B | 6864 | C | T | - | 5.09 |
| NS4B | 6871 | G | A | Gly16Arg | 4.96 |
| NS4B | 6879 | C | T | - | 5.90 |
| NS4B | 6943 | C | T | - | 2.71 |
| NS4B | 6948 | C | T | - | 5.19 |
| NS4B | 6951 | C | A | - | 1.45 |
| NS4B | 6982 | C | T | Arg53* | 1.42 |
| NS4B | 7041 | C | T | - | 4.01 |
| NS4B | 7059 | C | T | - | 4.29 |

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|---|---|---|---|---|---|
| E | 2223 | C | T | - | 2.58 |
| E | 2247 | C | T | - | 2.04 |
| E | 2256 | C | T | - | 6.74 |
| E | 2289 | T | C | - | 2.78 |
| E | 2307 | T | C | - | 2.02 |
| E | 2319 | A | C | - | 1.82 |
| NS1 | 2434 | G | A | Val5Ile | 4.33 |
| NS1 | 2448 | G | A | - | 6.01 |
| NS1 | 2451 | C | T | - | 4.15 |
| NS1 | 2484 | C | T | - | 4.74 |
| NS1 | 2494 | G | A | Val25Met | 2.74 |
| NS1 | 2511 | A | G | - | 3.83 |
| NS1 | 2560 | C | CA | Lys48fs | 0.98 |
| NS1 | 2589 | G | A | - | 3.58 |
| NS1 | 2781 | A | G | - | 2.60 |
| NS1 | 2878 | A | C | Met153Leu | 3.68 |
| NS1 | 2961 | T | C | - | 3.56 |
| NS1 | 2966 | A | G | Lys182Arg | 2.64 |
| NS1 | 2976 | G | A | - | 6.67 |
| NS1 | 2991 | C | T | - | 4.00 |
| NS1 | 3075 | C | T | - | 5.79 |
| NS1 | 3114 | C | A | - | 5.03 |
| NS1 | 3129 | A | G | - | 3.51 |
| NS1 | 3152 | C | CA | Asn246fs | 2.13 |
| NS1 | 3234 | C | T | - | 6.55 |
| NS1 | 3330 | T | C | - | 5.54 |
| NS1 | 3398 | A | G | Glu326Gly | 2.14 |
| NS1 | 3402 | C | T | - | 3.90 |
| NS1 | 3432 | C | T | - | 3.48 |
| NS1 | 3438 | G | A | - | 2.64 |
| NS1 | 3447 | G | A | - | 3.54 |
| NS2A | 3489 | G | A | - | 5.06 |
| NS2A | 3595 | T | C | Phe40Leu | 3.52 |
| NS2A | 3597 | T | C | - | 3.23 |
| NS2A | 3612 | T | A | - | 1.99 |
| NS2A | 3624 | C | T | - | 5.59 |
| NS2A | 3629 | A | G | Lys51Arg | 3.93 |
| NS2A | 3743 | C | T | Ala89Val | 4.36 |
| NS2A | 3750 | A | G | - | 2.08 |
| NS2A | 3753 | T | C | - | 5.05 |
| NS2A | 3768 | T | C | - | 4.54 |
| NS2A | 3858 | A | G | - | 3.66 |
| NS2A | 3864 | A | G | - | 2.43 |
| NS2A | 3875 | T | C | Val133Ala | 3.08 |
| NS2A | 3876 | C | T | - | 2.91 |
| NS2A | 3879 | A | C | - | 3.30 |
| NS2A | 3906 | C | T | - | 3.24 |
| NS2A | 3921 | C | T | - | 4.00 |
| NS2A | 3928 | G | C | Ala151Pro | 0.82 |
| NS2A | 4011 | C | T | - | 5.41 |
| NS2A | 4034 | G | A | Arg186Gln | 5.36 |
| NS2A | 4063 | T | C | - | 3.65 |
| NS2A | 4069 | G | A | Val198Ile | 12.79 |
| NS2A | 4083 | T | C | - | 3.53 |
| NS2A | 4110 | C | T | - | 4.83 |
| NS2B | 4164 | T | C | - | 2.58 |
| NS2B | 4206 | C | T | - | 2.12 |
| NS2B | 4350 | C | T | - | 5.56 |
| NS2B | 4414 | T | C | - | 9.84 |
| NS2B | 4443 | G | T | - | 8.18 |
| NS4B | 7062 | A | G | - | 3.59 |
| NS4B | 7083 | A | G | - | 11.59 |
| NS4B | 7092 | T | C | - | 11.85 |
| NS4B | 7137 | C | T | - | 4.88 |
| NS4B | 7161 | T | C | - | 4.25 |
| NS4B | 7165 | C | T | - | 7.25 |
| NS4B | 7248 | T | C | - | 22.54 |
| NS4B | 7314 | G | A | - | 6.84 |
| NS4B | 7356 | T | C | - | 5.94 |
| NS4B | 7383 | C | T | - | 3.55 |
| NS4B | 7428 | T | C | - | 2.55 |
| NS4B | 7449 | T | C | - | 4.09 |
| NS4B | 7518 | T | C | - | 5.05 |
| NS4B | 7551 | T | C | - | 7.84 |
| NS5 | 7656 | A | G | - | 26.87 |
| NS5 | 7776 | T | C | - | 14.58 |
| NS5 | 7812 | C | T | - | 6.56 |
| NS5 | 7911 | T | C | - | 3.68 |
| NS5 | 7996 | C | T | - | 8.68 |
| NS5 | 8049 | G | A | - | 4.88 |
| NS5 | 8083 | T | C | - | 4.61 |
| NS5 | 8103 | T | C | - | 3.22 |
| NS5 | 8121 | C | T | - | 3.00 |
| NS5 | 8143 | G | GA | Met194fs | 1.69 |
| NS5 | 8184 | G | A | - | 5.07 |
| NS5 | 8214 | G | A | - | 6.22 |
| NS5 | 8235 | T | C | - | 7.94 |
| NS5 | 8241 | C | T | - | 10.37 |
| NS5 | 8284 | C | T | - | 11.84 |
| NS5 | 8289 | T | C | - | 3.21 |
| NS5 | 8298 | T | C | - | 10.95 |
| NS5 | 8310 | C | T | - | 25.22 |
| NS5 | 8448 | T | C | - | 11.11 |
| NS5 | 8544 | T | C | - | 10.71 |
| NS5 | 8574 | C | T | - | 4.08 |
| NS5 | 8604 | C | T | - | 7.81 |
| NS5 | 8619 | G | A | - | 11.21 |
| NS5 | 8745 | G | A | - | 17.24 |
| NS5 | 8803 | A | G | Ile412Val | 11.58 |
| NS5 | 8823 | A | G | - | 10.37 |
| NS5 | 8926 | A | AC | Asn453fs | 1.78 |
| NS5 | 9042 | T | C | - | 9.94 |
| NS5 | 9066 | T | C | - | 5.23 |
| NS5 | 9079 | T | C | - | 3.70 |
| NS5 | 9132 | A | G | - | 6.52 |
| NS5 | 9294 | T | C | - | 37.50 |
| NS5 | 9303 | A | G | - | 14.29 |
| NS5 | 9322 | T | C | Ser585Pro | 34.62 |
| NS5 | 9424 | C | T | - | 4.67 |
| NS5 | 9477 | T | C | - | 12.05 |
| NS5 | 9501 | C | G | His644Gln | 3.24 |
| NS5 | 9576 | C | T | - | 7.42 |
| NS5 | 9628 | G | A | Val687Ile | 7.14 |
| NS5 | 9810 | T | C | - | 4.25 |
| NS5 | 9825 | C | T | - | 3.09 |
| NS5 | 9840 | T | C | - | 2.29 |
| NS5 | 9849 | A | G | - | 2.79 |
| NS5 | 9902 | C | G | Ala778Gly | 2.51 |
| NS5 | 10071 | A | T | Glu834Asp | 5.58 |
| NS5 | 10083 | T | C | - | 5.28 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2B | 4482 | A | G | - | 4.95 | NS5 | 10155 | T | C | - | 24.54 |
| NS3 | 4596 | T | C | - | 5.15 | NS5 | 10203 | A | G | - | 9.71 |
| NS3 | 4641 | T | C | - | 4.27 | 3UTR | 10280 | A | G | - | 10.34 |
| NS3 | 4662 | T | C | - | 4.65 | 3UTR | 10313 | A | G | - | 16.95 |
| NS3 | 4695 | G | A | - | 5.45 | 3UTR | 10389 | CA | C | - | 7.03 |
| NS3 | 4703 | G | A | Arg61Lys | 5.00 | 3UTR | 10408 | C | T | - | 4.46 |
| NS3 | 4707 | A | G | - | 15.52 | 3UTR | 10444 | T | TA | - | 2.88 |
| NS3 | 4812 | T | C | - | 3.16 | 3UTR | 10612 | C | CA | - | 6.90 |

**183**

DF

Primary

2.33E+05

1

| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
|---|---|---|---|---|---|
| 5UTR | 70 | G | GT | - | 2.68 |
| C | 201 | C | T | - | 1.28 |
| C | 312 | C | CA | Ser75fs | 2.01 |
| C | 336 | C | T | - | 2.23 |
| C | 384 | T | C | - | 2.39 |
| C | 387 | A | G | - | 2.52 |
| prM/M | 445 | C | T | - | 2.85 |
| prM/M | 450 | T | C | - | 3.67 |
| prM/M | 457 | A | C | Asn7His | 2.44 |
| prM/M | 505 | CT | C | Leu24fs | 0.64 |
| prM/M | 508 | T | C | - | 2.75 |
| prM/M | 567 | A | G | - | 1.34 |
| prM/M | 737 | G | A | Gly100Glu | 1.66 |
| prM/M | 792 | A | G | - | 2.04 |
| prM/M | 798 | C | T | - | 1.38 |
| prM/M | 852 | A | G | - | 1.31 |
| prM/M | 856 | T | C | - | 1.27 |
| prM/M | 864 | T | C | - | 1.08 |
| prM/M | 873 | C | A | - | 1.76 |
| prM/M | 894 | A | C | - | 2.34 |
| prM/M | 912 | A | G | - | 4.14 |
| prM/M | 921 | C | T | - | 2.55 |
| E | 954 | C | A | - | 4.28 |
| E | 1005 | A | C | - | 3.86 |
| E | 1040 | C | CA | Asn37fs | 2.11 |
| E | 1102 | T | C | - | 4.11 |
| E | 1293 | C | T | - | 2.39 |
| E | 1311 | G | A | Met125Ile | 2.21 |
| E | 1860 | T | C | - | 4.23 |
| E | 1863 | A | G | - | 2.34 |
| E | 1963 | GA | G | Arg345fs | 0.73 |
| E | 1994 | C | CGATTG | Val354fs | 2.69 |
| E | 2055 | C | T | - | 2.07 |
| E | 2223 | C | T | - | 2.41 |
| E | 2247 | C | T | - | 1.31 |
| E | 2256 | C | T | - | 0.98 |
| E | 2276 | C | T | Ala447Val | 1.65 |
| E | 2289 | T | C | - | 1.90 |
| E | 2307 | T | C | - | 2.00 |
| E | 2308 | C | T | Leu458Phe | 0.94 |
| E | 2319 | A | C | - | 2.82 |
| E | 2328 | A | T | - | 1.95 |
| NS1 | 2434 | G | A | Val5Ile | 1.86 |
| NS1 | 2448 | G | A | - | 1.81 |
| NS3 | 4707 | A | G | - | 5.49 |
| NS3 | 4854 | G | A | - | 2.45 |
| NS3 | 4875 | C | T | - | 3.10 |
| NS3 | 4878 | C | T | - | 1.30 |
| NS3 | 4956 | A | G | - | 1.94 |
| NS3 | 4974 | C | T | - | 1.71 |
| NS3 | 5026 | G | GA | Ser171fs | 5.68 |
| NS3 | 5190 | T | C | - | 1.73 |
| NS3 | 5460 | C | T | - | 2.24 |
| NS3 | 5531 | G | T | Arg337Ile | 2.26 |
| NS3 | 5796 | T | C | - | 2.67 |
| NS3 | 5802 | T | C | - | 3.80 |
| NS3 | 5835 | G | A | - | 4.04 |
| NS3 | 5847 | T | C | - | 3.06 |
| NS3 | 5915 | C | CA | Asn467fs | 12.70 |
| NS3 | 5922 | C | T | - | 4.88 |
| NS3 | 6009 | C | T | - | 2.08 |
| NS3 | 6094 | C | T | - | 2.82 |
| NS3 | 6300 | T | C | - | 3.74 |
| NS3 | 6330 | C | T | - | 2.83 |
| NS4A | 6378 | C | T | - | 1.06 |
| NS4A | 6418 | A | G | Thr15Ala | 1.77 |
| NS4A | 6462 | C | T | - | 3.30 |
| NS4A | 6471 | T | C | - | 3.70 |
| NS4A | 6491 | G | A | Arg39Lys | 4.66 |
| NS4A | 6496 | C | T | His41Tyr | 4.72 |
| NS4A | 6500 | A | C | Asn42Thr | 3.98 |
| NS4A | 6528 | C | T | - | 3.56 |
| NS4A | 6547 | T | C | - | 5.44 |
| NS4A | 6556 | T | C | - | 5.14 |
| NS4A | 6562 | A | G | Thr63Ala | 4.53 |
| NS4A | 6564 | A | G | - | 4.36 |
| NS4A | 6585 | A | G | - | 4.22 |
| NS4A | 6633 | C | T | - | 3.59 |
| NS4A | 6654 | C | T | - | 3.42 |
| NS4A | 6681 | G | A | - | 6.30 |
| NS4A | 6711 | G | GT | Leu115fs | 1.66 |
| NS4B | 6864 | C | T | - | 2.30 |
| NS4B | 6871 | G | A | Gly16Arg | 2.92 |
| NS4B | 6879 | C | T | - | 2.57 |
| NS4B | 6948 | C | T | - | 1.05 |
| NS4B | 6982 | C | T | Arg53* | 1.47 |
| NS4B | 7008 | G | A | - | 1.66 |
| NS4B | 7029 | T | A | - | 1.72 |
| NS4B | 7041 | C | T | - | 2.21 |
| NS4B | 7059 | C | T | - | 3.03 |
| NS4B | 7062 | A | G | - | 1.74 |
| NS4B | 7083 | A | G | - | 4.75 |
| NS4B | 7137 | C | T | - | 1.97 |
| NS4B | 7248 | T | C | - | 4.42 |

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|---|---|---|---|---|---|
| NS1 | 2451 | C | T | - | 1.26 |
| NS1 | 2484 | C | T | - | 1.79 |
| NS1 | 2511 | A | G | - | 1.10 |
| NS1 | 2560 | A | C | Lys47Gln | 2.79 |
| NS1 | 2589 | G | A | - | 1.47 |
| NS1 | 2781 | A | G | - | 3.21 |
| NS1 | 2878 | A | C | Met153Leu | 3.43 |
| NS1 | 2898 | C | T | - | 1.59 |
| NS1 | 2976 | G | A | - | 1.49 |
| NS1 | 2991 | C | T | - | 1.55 |
| NS1 | 3075 | C | T | - | 1.53 |
| NS1 | 3234 | C | T | - | 0.88 |
| NS1 | 3330 | T | C | - | 2.27 |
| NS1 | 3398 | A | G | Glu326Gly | 2.10 |
| NS1 | 3402 | C | T | - | 1.39 |
| NS2A | 3624 | C | T | - | 1.52 |
| NS2A | 3629 | A | G | Lys51Arg | 1.51 |
| NS2A | 3720 | C | CA | Val83fs | 0.49 |
| NS2A | 3753 | T | C | - | 2.47 |
| NS2A | 3768 | T | C | - | 2.40 |
| NS2A | 3864 | A | G | - | 1.87 |
| NS2A | 3866 | G | C | Gly130Ala | 1.44 |
| NS2A | 3875 | T | C | Val133Ala | 2.01 |
| NS2A | 3876 | C | T | - | 2.01 |
| NS2A | 3879 | A | C | - | 1.94 |
| NS2A | 3921 | C | T | - | 2.17 |
| NS2A | 3928 | G | C | Ala151Pro | 1.16 |
| NS2A | 4011 | C | T | - | 2.86 |
| NS2A | 4034 | G | A | Arg186Gln | 4.16 |
| NS2A | 4063 | T | C | - | 3.04 |
| NS2A | 4069 | G | A | Val198Ile | 4.73 |
| NS2A | 4083 | T | C | - | 2.59 |
| NS2A | 4110 | C | T | - | 1.33 |
| NS2B | 4482 | A | G | - | 3.27 |
| NS2B | 4488 | G | A | - | 5.45 |
| NS2B | 4491 | G | A | - | 2.00 |
| NS4B | 7314 | G | A | - | 2.88 |
| NS4B | 7383 | C | T | - | 2.00 |
| NS5 | 7911 | T | C | - | 2.75 |
| NS5 | 7959 | T | C | - | 1.88 |
| NS5 | 7996 | C | T | - | 2.38 |
| NS5 | 7999 | T | C | - | 1.06 |
| NS5 | 8049 | G | A | - | 0.80 |
| NS5 | 8074 | G | C | Glu169Gln | 2.32 |
| NS5 | 8104 | T | G | Cys179Gly | 0.97 |
| NS5 | 8121 | C | T | - | 1.06 |
| NS5 | 8184 | G | A | - | 3.74 |
| NS5 | 8241 | C | T | - | 6.17 |
| NS5 | 8284 | C | T | - | 5.17 |
| NS5 | 8298 | T | C | - | 4.26 |
| NS5 | 8310 | C | T | - | 5.19 |
| NS5 | 8544 | T | C | - | 1.84 |
| NS5 | 8574 | C | T | - | 1.47 |
| NS5 | 8803 | A | G | Ile412Val | 3.42 |
| NS5 | 8926 | A | AC | Asn453fs | 3.01 |
| NS5 | 8967 | T | C | - | 2.26 |
| NS5 | 9042 | T | C | - | 1.60 |
| NS5 | 9066 | T | C | - | 3.07 |
| NS5 | 9079 | T | C | - | 2.08 |
| NS5 | 9208 | C | T | - | 27.27 |
| NS5 | 9226 | A | G | Ile553Val | 27.27 |
| NS5 | 9322 | T | C | Ser585Pro | 8.33 |
| NS5 | 9477 | T | C | - | 5.98 |
| NS5 | 9576 | C | T | - | 4.29 |
| NS5 | 9628 | G | A | Val687Ile | 5.00 |
| NS5 | 9795 | A | G | - | 3.88 |
| NS5 | 9825 | C | T | - | 2.78 |
| NS5 | 9840 | T | C | - | 2.62 |
| NS5 | 9849 | A | G | - | 4.23 |
| NS5 | 9902 | C | G | Ala778Gly | 1.22 |
| 3UTR | 10408 | C | T | - | 1.83 |
| 3UTR | 10444 | T | TA | - | 1.64 |

| **185** | | | | | |
|---|---|---|---|---|---|
| WS | | | | | |
| Secondary | | | | | |
| 6.47E+05 | | | | | |
| 2 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 70 | G | GT | - | 0.85 |
| C | 135 | C | T | - | 2.18 |
| C | 341 | G | T | Arg82Ile | 0.53 |
| prM/M | 522 | G | A | - | 0.91 |
| prM/M | 628 | A | G | Ile64Val | 0.97 |
| prM/M | 650 | C | T | Thr71Met | 0.49 |
| prM/M | 703 | G | GA | Arg91fs | 2.16 |
| prM/M | 837 | T | C | - | 0.66 |
| E | 1001 | A | C | Asp22Ala | 0.91 |
| E | 1040 | C | CA | Asn37fs | 2.67 |
| E | 1385 | C | G | Ala150Gly | 0.73 |
| E | 1390 | G | C | Gly152Arg | 0.73 |
| E | 1695 | A | G | - | 1.53 |
| NS1 | 2756 | A | G | Lys112Arg | 0.68 |
| NS1 | 2969 | T | A | Leu183His | 1.13 |
| NS1 | 3074 | T | A | Ile218Asn | 0.93 |
| NS3 | 5717 | C | T | Ala399Val | 1.04 |
| NS3 | 5915 | C | CA | Asn467fs | 4.12 |
| NS3 | 6083 | G | T | Gly521Val | 0.54 |
| NS3 | 6155 | G | A | Trp545* | 0.65 |
| NS3 | 6285 | G | A | - | 0.98 |
| NS4A | 6625 | G | A | Gly84Arg | 1.46 |
| NS4A | 6790 | A | T | Ile139Leu | 1.50 |
| NS4B | 6844 | G | GA | Thr9fs | 1.67 |
| NS4B | 6844 | G | A | Glu7Lys | 1.28 |
| NS4B | 6922 | C | T | Arg33Cys | 0.66 |
| NS4B | 6982 | C | T | Arg53* | 1.62 |
| NS4B | 7049 | T | C | Leu75Ser | 0.61 |
| NS4B | 7071 | G | T | Trp82Cys | 0.54 |
| NS4B | 7152 | A | T | - | 0.81 |
| NS4B | 7435 | C | G | Leu204Val | 0.60 |
| NS5 | 7784 | C | T | Thr72Ile | 0.61 |
| NS5 | 8103 | C | T | - | 0.54 |
| NS5 | 8204 | G | T | Arg212Leu | 1.17 |
| NS5 | 8256 | G | T | - | 0.85 |
| NS5 | 8891 | T | TA | His442fs | 1.43 |
| NS5 | 8926 | A | AC | Asn453fs | 1.64 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| NS1 | 3398 | A | G | Glu326Gly | 0.95 | NS5 | 9432 | A | G | - | 1.93 |
| NS2A | 3600 | G | T | - | 1.14 | NS5 | 9705 | T | TC | Phe713fs | 1.41 |
| NS2B | 4443 | G | T | - | 1.52 | NS5 | 9714 | G | C | Glu715Asp | 0.63 |
| NS3 | 4826 | C | T | Pro102Leu | 0.55 | NS5 | 9718 | G | C | Val717Leu | 0.56 |
| NS3 | 4837 | C | T | Pro106Ser | 1.12 | NS5 | 9726 | AG | A | Asp720fs | 0.64 |
| NS3 | 5304 | T | G | Cys261Trp | 1.42 | NS5 | 9902 | C | G | Ala778Gly | 3.00 |
| NS3 | 5309 | C | A | Ala263Asp | 0.98 | 3UTR | 10611 | C | CA | - | 23.90 |
| NS3 | 5433 | A | T | - | 2.46 | 3UTR | 10611 | C | CAA | - | 4.50 |
| NS3 | 5436 | G | A | - | 2.64 | 3UTR | 10611 | CAA | C | - | 11.50 |
| NS3 | 5531 | G | T | Arg337Ile | 0.91 | 3UTR | 10611 | CA | C | - | 1.20 |
| NS3 | 5642 | G | T | Cys374Phe | 0.73 | 3UTR | 10629 | C | T | - | 0.77 |

| **186** | | | | | | NS3 | 4829 | G | GA | Asn105fs | 0.65 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WS | | | | | | NS3 | 4829 | G | A | Gly103Glu | 0.65 |
| Primary | | | | | | NS3 | 4837 | C | T | Pro106Ser | 2.17 |
| 1.62E+04 | | | | | | NS3 | 4854 | A | G | - | 0.91 |
| 3 | | | | | | NS3 | 4905 | G | A | - | 0.97 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5026 | G | A | Glu169Lys | 0.86 |
| 5UTR | 70 | G | GT | - | 2.88 | NS3 | 5026 | G | GA | Ser171fs | 5.07 |
| C | 135 | C | T | - | 1.51 | NS3 | 5135 | C | A | Pro205Gln | 0.55 |
| C | 201 | T | C | - | 0.68 | NS3 | 5235 | C | T | - | 0.96 |
| prM/M | 522 | G | A | - | 0.62 | NS3 | 5309 | C | A | Ala263Asp | 0.64 |
| prM/M | 567 | G | A | - | 0.81 | NS3 | 5433 | A | T | - | 1.95 |
| prM/M | 628 | A | G | Ile64Val | 1.15 | NS3 | 5436 | G | A | - | 2.00 |
| prM/M | 650 | C | T | Thr71Met | 1.02 | NS3 | 5451 | C | A | - | 0.98 |
| prM/M | 792 | G | A | - | 0.82 | NS3 | 5460 | T | C | - | 1.23 |
| prM/M | 837 | T | C | - | 1.45 | NS3 | 5531 | G | T | Arg337Ile | 1.24 |
| prM/M | 852 | G | A | - | 0.95 | NS3 | 5648 | G | GA | Lys377fs | 2.28 |
| prM/M | 856 | C | T | - | 0.91 | NS3 | 5716 | G | A | Ala399Thr | 2.63 |
| prM/M | 864 | C | T | - | 0.86 | NS3 | 5751 | C | T | - | 1.09 |
| prM/M | 873 | A | C | - | 0.81 | NS3 | 5754 | G | A | - | 0.95 |
| prM/M | 894 | C | A | - | 1.03 | NS3 | 5802 | C | T | - | 0.60 |
| prM/M | 921 | T | C | - | 0.90 | NS3 | 5887 | C | T | Gln456* | 1.76 |
| E | 1001 | A | C | Asp22Ala | 0.68 | NS3 | 5915 | C | CA | Asn467fs | 6.04 |
| E | 1590 | G | A | - | 0.69 | NS3 | 6009 | T | C | - | 0.91 |
| E | 1695 | A | G | - | 0.75 | NS3 | 6069 | G | T | - | 0.95 |
| E | 1963 | G | GA | Arg345fs | 0.58 | NS3 | 6285 | G | A | - | 2.65 |
| E | 2007 | T | C | - | 1.49 | NS3 | 6293 | T | TA | Lys592fs | 0.49 |
| E | 2023 | A | G | Ser363Gly | 1.05 | NS4A | 6471 | C | T | - | 0.80 |
| E | 2024 | G | C | | 0.96 | NS4A | 6625 | G | A | Gly84Arg | 1.12 |
| E | 2049 | C | T | - | 0.71 | NS4A | 6790 | A | T | Ile139Leu | 0.85 |
| E | 2064 | T | C | - | 0.93 | NS4B | 6844 | G | GA | Thr9fs | 1.99 |
| E | 2067 | C | T | - | 0.83 | NS4B | 6844 | G | A | Glu7Lys | 2.48 |
| E | 2073 | C | T | - | 1.34 | NS4B | 6922 | C | T | Arg33Cys | 0.65 |
| E | 2210 | T | A | Leu425* | 1.61 | NS4B | 6982 | C | T | Arg53* | 1.60 |
| E | 2349 | T | C | - | 5.14 | NS4B | 7049 | T | C | Leu75Ser | 0.90 |
| NS1 | 2434 | A | G | Ile5Val | 1.51 | NS4B | 7062 | G | A | - | 0.55 |
| NS1 | 2448 | A | G | - | 1.03 | NS4B | 7356 | C | T | - | 0.66 |
| NS1 | 2451 | T | C | - | 1.26 | NS4B | 7518 | C | T | - | 1.22 |
| NS1 | 2484 | T | C | - | 0.91 | NS5 | 7600 | GA | G | Glu11fs | 0.72 |
| NS1 | 2511 | G | A | - | 0.86 | NS5 | 7656 | GA | G | Ser31fs | 0.51 |
| NS1 | 2589 | A | G | - | 1.15 | NS5 | 7959 | C | T | - | 0.75 |
| NS1 | 2756 | A | G | Lys112Arg | 0.97 | NS5 | 8103 | C | T | - | 1.00 |
| NS1 | 2781 | G | A | - | 0.89 | NS5 | 8104 | T | G | Cys179Gly | 1.31 |
| NS1 | 2991 | T | C | - | 0.90 | NS5 | 8143 | G | GA | Met194fs | 1.04 |
| NS1 | 3152 | C | CA | Asn246fs | 4.99 | NS5 | 8184 | A | G | - | 0.74 |
| NS1 | 3408 | C | T | - | 0.59 | NS5 | 8204 | G | T | Arg212Leu | 0.78 |
| NS1 | 3438 | A | G | - | 1.03 | NS5 | 8678 | CA | C | Lys371fs | 1.06 |
| NS1 | 3447 | A | G | - | 1.25 | | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| NS1 | 3474 | A | G | - | 2.40 |
| NS2A | 3552 | C | T | - | 2.59 |
| NS2A | 3600 | G | T | - | 0.95 |
| NS2A | 3624 | T | C | - | 3.09 |
| NS2A | 3629 | G | A | Arg51Lys | 1.29 |
| NS2A | 3753 | C | T | - | 0.78 |
| NS2A | 3768 | C | T | - | 0.78 |
| NS2A | 3864 | G | A | - | 0.65 |
| NS2A | 3875 | C | T | Ala133Val | 0.69 |
| NS2A | 3879 | C | A | - | 0.64 |
| NS2A | 3921 | T | C | - | 1.04 |
| NS2A | 4011 | T | C | - | 1.03 |
| NS2A | 4063 | C | T | - | 1.39 |
| NS2A | 4083 | C | T | - | 0.92 |
| NS2A | 4099 | C | T | - | 2.01 |
| NS2B | 4164 | C | T | - | 0.54 |
| NS5 | 8891 | T | TA | His442fs | 0.73 |
| NS5 | 8926 | A | AC | Asn453fs | 1.68 |
| NS5 | 8967 | C | T | - | 1.01 |
| NS5 | 9038 | G | T | Gly490Val | 0.63 |
| NS5 | 9209 | T | TA | Asn549fs | 1.43 |
| NS5 | 9491 | C | T | Thr641Ile | 0.86 |
| NS5 | 9628 | A | G | Ile687Val | 0.94 |
| NS5 | 9705 | T | TC | Phe713fs | 0.90 |
| NS5 | 9825 | T | C | - | 1.24 |
| NS5 | 9840 | C | T | - | 1.19 |
| NS5 | 9849 | G | A | - | 1.05 |
| NS5 | 9902 | C | G | Ala778Gly | 1.38 |
| NS5 | 10083 | C | T | - | 0.68 |
| NS5 | 10202 | G | A | Gly878Glu | 1.57 |
| NS5 | 10252 | G | A | Glu895Lys | 4.15 |
| 3UTR | 10565 | G | C | - | 1.12 |

**187**

WS

Secondary

6.19E+04

3

| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
|---|---|---|---|---|---|
| 5UTR | 70 | G | GT | - | 1.16 |
| C | 135 | C | T | - | 1.10 |
| prM/M | 522 | G | A | - | 0.76 |
| prM/M | 703 | G | GA | Arg91fs | 2.23 |
| prM/M | 734 | T | TGGAACAAGC | Val99Gly100insGluGlnAla | 0.73 |
| E | 1001 | A | C | Asp22Ala | 0.87 |
| E | 1695 | A | G | - | 1.09 |
| E | 2073 | C | T | - | 0.90 |
| E | 2210 | T | A | Leu425* | 1.02 |
| NS1 | 3074 | T | A | Ile218Asn | 0.96 |
| NS1 | 3152 | C | CA | Asn246fs | 4.87 |
| NS2A | 3600 | G | T | - | 0.83 |
| NS2B | 4443 | G | T | - | 1.22 |
| NS3 | 4829 | G | GA | Asn105fs | 0.56 |
| NS3 | 5433 | A | T | - | 3.20 |
| NS3 | 5436 | G | A | - | 3.15 |
| NS3 | 5717 | C | T | Ala399Val | 1.98 |
| NS3 | 5887 | C | T | Gln456* | 1.03 |
| NS3 | 5915 | C | CA | Asn467fs | 5.23 |
| NS3 | 6145 | T | C | - | 13.27 |
| NS3 | 6285 | G | A | - | 1.16 |
| NS3 | 6285 | G | GA | Lys590fs | 1.24 |
| NS3 | 6293 | T | TA | Lys592fs | 0.57 |
| NS4A | 6625 | G | A | Gly84Arg | 1.08 |
| NS4A | 6790 | A | T | Ile139Leu | 0.84 |
| NS4B | 6844 | G | GA | Thr9fs | 1.65 |
| NS4B | 6844 | G | A | Glu7Lys | 1.21 |
| NS4B | 6888 | G | A | - | 0.51 |
| NS4B | 6922 | C | T | Arg33Cys | 0.92 |
| NS4B | 6982 | C | T | Arg53* | 1.21 |
| NS4B | 7049 | T | C | Leu75Ser | 0.64 |
| NS5 | 8143 | G | GA | Met194fs | 0.92 |
| NS5 | 8184 | G | A | - | 43.80 |
| NS5 | 8256 | G | T | - | 0.77 |
| NS5 | 8499 | A | T | Glu310Asp | 1.27 |
| NS5 | 8891 | T | TA | His442fs | 1.20 |
| NS5 | 8926 | A | AC | Asn453fs | 1.29 |
| NS5 | 9705 | T | TC | Phe713fs | 0.89 |
| NS5 | 9714 | G | C | Glu715Asp | 0.69 |
| NS5 | 9718 | G | C | Val717Leu | 0.67 |
| NS5 | 9726 | AG | A | Asp720fs | 0.58 |
| NS5 | 9902 | C | G | Ala778Gly | 2.48 |

**188**

WS

Primary

1.32E+06

2

| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
|---|---|---|---|---|---|
| 5UTR | 70 | G | GT | - | 1.70 |
| 5UTR | 96 | G | A | - | 1.34 |
| C | 135 | C | T | - | 4.90 |
| C | 148 | C | G | Arg18Gly | 0.81 |
| C | 341 | G | T | Arg82Ile | 1.84 |
| prM/M | 628 | A | T | Ile64Leu | 0.70 |
| prM/M | 808 | G | A | Glu124Lys | 0.79 |
| E | 1001 | A | C | Asp22Ala | 1.07 |
| E | 1039 | G | T | Ala35Ser | 0.63 |
| E | 1311 | G | A | Met125Ile | 0.54 |
| NS3 | 5436 | G | A | - | 3.33 |
| NS3 | 5531 | G | T | Arg337Ile | 1.12 |
| NS3 | 5642 | G | T | Cys374Phe | 0.59 |
| NS3 | 5717 | C | T | Ala399Val | 0.93 |
| NS3 | 5887 | C | T | Gln456* | 0.96 |
| NS3 | 6033 | T | A | - | 0.71 |
| NS3 | 6285 | G | A | - | 0.75 |
| NS3 | 6293 | T | TA | Lys592fs | 0.47 |
| NS4A | 6625 | G | A | Gly84Arg | 1.29 |
| NS4B | 6844 | G | A | Glu7Lys | 1.13 |
| NS4B | 6894 | T | C | - | 0.99 |
| NS4B | 6922 | C | T | Arg33Cys | 1.11 |
| NS4B | 6958 | A | G | Thr45Ala | 0.93 |
| NS4B | 7049 | T | C | Leu75Ser | 0.71 |
| NS4B | 7071 | G | T | Trp82Cys | 0.52 |
| NS4B | 7152 | A | T | - | 0.96 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1359 | A | G | Ile141Met | 0.77 | NS4B | 7435 | C | G | Leu204Val | 0.61 |
| E | 1396 | A | G | Asn154Asp | 19.85 | NS5 | 7600 | GA | G | Glu11fs | 0.53 |
| E | 1695 | A | G | - | 0.87 | NS5 | 7645 | C | A | Gln26Lys | 0.91 |
| E | 1842 | T | C | - | 16.17 | NS5 | 7784 | C | T | Thr72Ile | 0.80 |
| E | 1915 | G | A | Glu327Lys | 0.48 | NS5 | 8074 | G | C | Glu169Gln | 0.83 |
| E | 2210 | T | A | Leu425* | 1.37 | NS5 | 8204 | G | T | Arg212Leu | 0.78 |
| NS1 | 2756 | A | G | Lys112Arg | 0.90 | NS5 | 8256 | G | T | - | 0.99 |
| NS1 | 2911 | A | T | Thr164Ser | 12.83 | NS5 | 8848 | G | C | Glu427Gln | 0.55 |
| NS1 | 3012 | T | C | - | 0.84 | NS5 | 8926 | A | AC | Asn453fs | 2.34 |
| NS1 | 3053 | A | C | Lys211Thr | 0.79 | NS5 | 8932 | A | T | Met455Leu | 1.01 |
| NS2A | 3877 | C | A | Leu134Ile | 1.08 | NS5 | 9705 | T | TC | Phe713fs | 1.17 |
| NS2B | 4443 | G | T | - | 1.50 | NS5 | 9714 | G | C | Glu715Asp | 0.85 |
| NS3 | 4635 | C | T | - | 0.46 | NS5 | 9716 | T | A | Leu716* | 0.83 |
| NS3 | 4829 | G | A | Gly103Glu | 0.59 | NS5 | 9718 | G | C | Val717Leu | 0.83 |
| NS3 | 4837 | C | T | Pro106Ser | 0.94 | NS5 | 9727 | G | A | Asp720Asn | 0.46 |
| NS3 | 5136 | A | T | - | 0.63 | NS5 | 9902 | C | G | Ala778Gly | 3.37 |
| NS3 | 5304 | T | G | Cys261Trp | 1.00 | NS5 | 10205 | A | C | Tyr879Ser | 1.69 |
| NS3 | 5309 | C | A | Ala263Asp | 0.58 | 3UTR | 10500 | C | A | - | 0.48 |
| NS3 | 5433 | A | T | - | 2.90 | 3UTR | 10629 | C | T | - | 0.49 |

| **189** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WS | | | | | | NS3 | 5460 | T | C | - | 2.98 |
| Primary | | | | | | NS3 | 5526 | G | A | - | 1.13 |
| 1.21E+05 | | | | | | NS3 | 5648 | G | GA | Lys377fs | 2.43 |
| 1 | | | | | | NS3 | 5717 | C | T | Ala399Val | 1.20 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5802 | C | T | - | 0.57 |
| 5UTR | 70 | G | GT | - | 0.96 | NS3 | 5887 | C | T | Gln456* | 0.76 |
| C | 110 | G | GA | Lys6fs | 0.59 | NS3 | 6285 | G | GA | Lys590fs | 1.93 |
| C | 135 | C | T | - | 1.39 | NS4A | 6625 | G | A | Gly84Arg | 1.03 |
| C | 148 | C | G | Arg18Gly | 0.60 | NS4A | 6790 | A | T | Ile139Leu | 1.07 |
| prM/M | 522 | G | A | - | 1.04 | NS4B | 6844 | G | A | Glu7Lys | 1.61 |
| prM/M | 703 | G | GA | Arg91fs | 2.59 | NS4B | 6922 | C | T | Arg33Cys | 0.92 |
| prM/M | 837 | T | C | - | 1.30 | NS4B | 6982 | C | T | Arg53* | 1.57 |
| E | 1001 | A | C | Asp22Ala | 0.84 | NS4B | 7041 | T | C | - | 0.72 |
| E | 1005 | C | A | - | 0.61 | NS4B | 7049 | T | C | Leu75Ser | 0.87 |
| E | 1293 | T | C | - | 1.36 | NS4B | 7062 | G | A | - | 0.71 |
| E | 1695 | A | G | - | 1.25 | NS4B | 7146 | C | A | - | 0.79 |
| E | 1959 | T | C | - | 35.45 | NS4B | 7429 | TC | T | Thr203fs | 0.19 |
| E | 1995 | A | G | - | 0.84 | NS4B | 7471 | A | G | Thr216Ala | 1.39 |
| E | 2014 | G | GA | Asp362fs | 0.37 | NS4B | 7518 | C | T | - | 1.16 |
| E | 2023 | A | G | Ser363Gly | 1.34 | NS5 | 7600 | GA | G | Glu11fs | 1.04 |
| E | 2024 | G | C | | 1.31 | NS5 | 7776 | C | T | - | 3.04 |
| E | 2049 | C | T | - | 1.93 | NS5 | 7812 | T | C | - | 5.37 |
| E | 2064 | T | C | - | 1.74 | NS5 | 7911 | C | T | - | 1.23 |
| E | 2067 | C | T | - | 1.54 | NS5 | 7959 | C | T | - | 0.94 |
| E | 2073 | C | T | - | 1.81 | NS5 | 8049 | A | G | - | 1.16 |
| E | 2210 | T | A | Leu425* | 1.55 | NS5 | 8103 | C | T | - | 0.77 |
| NS1 | 2756 | A | G | Lys112Arg | 1.08 | NS5 | 8104 | T | G | Cys179Gly | 1.43 |
| NS2A | 3489 | G | A | - | 1.19 | NS5 | 8204 | G | T | Arg212Leu | 1.22 |
| NS2A | 3549 | G | A | - | 9.26 | NS5 | 8256 | G | T | - | 0.59 |
| NS2A | 3552 | C | T | - | 2.79 | NS5 | 8284 | T | C | - | 1.10 |
| NS2A | 3600 | G | T | - | 1.06 | NS5 | 8298 | C | T | - | 1.23 |
| NS2A | 3624 | T | C | - | 7.53 | NS5 | 8319 | T | C | - | 9.67 |
| NS2A | 4011 | T | C | - | 0.79 | NS5 | 8544 | C | T | - | 0.81 |
| NS2A | 4034 | A | G | Gln186Arg | 0.71 | NS5 | 8803 | G | A | Val412Ile | 0.73 |
| NS2A | 4063 | C | T | - | 0.82 | NS5 | 8891 | T | TA | His442fs | 1.65 |
| NS2A | 4083 | C | T | - | 0.68 | NS5 | 8919 | TG | T | Val451fs | 0.70 |
| NS2B | 4164 | C | T | - | 0.54 | NS5 | 8926 | A | AC | Asn453fs | 1.60 |
| NS3 | 4829 | G | GA | Asn105fs | 0.50 | NS5 | 9132 | G | A | - | 1.75 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS3 | 4837 | C | T | Pro106Ser | 1.10 | NS5 | 9705 | T | TC | Phe713fs | 0.86 |
| NS3 | 5026 | G | GA | Ser171fs | 4.72 | NS5 | 9714 | G | C | Glu715Asp | 0.62 |
| NS3 | 5070 | C | T | - | 0.69 | NS5 | 9726 | AG | A | Asp720fs | 0.45 |
| NS3 | 5120 | CA | C | Lys201fs | 0.24 | NS5 | 9902 | C | G | Ala778Gly | 2.12 |
| NS3 | 5190 | C | T | - | 0.51 | NS5 | 9909 | CT | C | Ser781fs | 0.26 |
| NS3 | 5409 | T | A | - | 1.68 | NS5 | 10155 | T | C | - | 2.15 |
| NS3 | 5433 | A | T | - | 3.58 | NS5 | 10202 | G | A | Gly878Glu | 1.19 |
| NS3 | 5436 | G | A | - | 3.26 | 3UTR | 10443 | T | TA | - | 1.55 |
| NS3 | 5451 | C | A | - | 3.74 | 3UTR | 10599 | A | AC | - | 0.80 |

| **190** | | | | | | NS3 | 5304 | C | T | - | 2.88 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WS | | | | | | NS3 | 5382 | C | T | - | 1.52 |
| Secondary | | | | | | NS3 | 5415 | A | G | - | 4.22 |
| 9.89E+04 | | | | | | NS3 | 5649 | G | GA | Asn378fs | 0.92 |
| 2 | | | | | | NS3 | 5649 | G | A | - | 3.99 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5717 | T | C | Val399Ala | 6.78 |
| 5UTR | 70 | G | GT | - | 4.39 | NS3 | 5730 | C | T | - | 1.93 |
| C | 135 | C | T | - | 2.88 | NS3 | 5918 | G | A | Arg466Lys | 3.87 |
| C | 309 | G | A | - | 1.72 | NS3 | 6285 | G | GA | Lys590fs | 1.19 |
| C | 312 | C | CA | Ser75fs | 4.37 | NS4A | 6711 | G | GT | Leu115fs | 3.96 |
| C | 336 | T | C | - | 3.53 | NS4B | 6844 | G | GA | Thr9fs | 1.00 |
| prM/M | 457 | A | C | Asn7His | 1.82 | NS4B | 6844 | G | A | Glu7Lys | 2.12 |
| prM/M | 606 | C | T | - | 1.39 | NS4B | 6922 | C | T | Arg33Cys | 1.84 |
| prM/M | 703 | G | GA | Arg91fs | 4.21 | NS4B | 6943 | C | T | - | 1.93 |
| prM/M | 802 | A | G | Arg122Gly | 1.34 | NS4B | 6958 | A | G | Thr45Ala | 1.29 |
| prM/M | 847 | G | A | Ala137Thr | 1.11 | NS4B | 6982 | C | T | Arg53* | 2.22 |
| prM/M | 893 | T | C | Val152Ala | 3.16 | NS4B | 7059 | C | T | - | 1.20 |
| prM/M | 912 | G | A | - | 1.34 | NS4B | 7083 | G | A | - | 1.58 |
| E | 954 | C | A | - | 2.93 | NS4B | 7092 | C | T | - | 2.03 |
| E | 1040 | C | CA | Asn37fs | 3.91 | NS4B | 7134 | C | T | - | 1.24 |
| E | 1110 | A | G | - | 9.77 | NS4B | 7137 | T | C | - | 3.69 |
| E | 1590 | A | G | - | 11.19 | NS4B | 7413 | G | A | - | 5.23 |
| E | 1860 | C | T | - | 7.94 | NS4B | 7428 | T | C | - | 18.90 |
| E | 1963 | G | GA | Arg345fs | 1.08 | NS4B | 7497 | T | C | - | 5.34 |
| E | 1994 | C | CGATTG | Val354fs | 1.27 | NS5 | 7632 | A | G | - | 4.79 |
| E | 2220 | A | G | - | 3.07 | NS5 | 7650 | C | T | - | 6.49 |
| E | 2256 | C | T | - | 2.43 | NS5 | 7656 | G | A | - | 4.24 |
| E | 2276 | C | T | Ala447Val | 1.32 | NS5 | 7945 | T | C | - | 5.45 |
| E | 2319 | A | C | - | 2.18 | NS5 | 7996 | T | C | - | 3.10 |
| E | 2393 | T | C | Ile486Thr | 3.24 | NS5 | 8074 | G | C | Glu169Gln | 1.54 |
| NS1 | 2494 | G | A | Val25Met | 1.46 | NS5 | 8184 | G | A | - | 1.51 |
| NS1 | 2617 | T | C | - | 5.92 | NS5 | 8241 | C | T | - | 1.79 |
| NS1 | 2889 | G | A | - | 3.70 | NS5 | 8298 | T | C | - | 2.40 |
| NS1 | 2961 | T | C | - | 2.35 | NS5 | 8544 | T | C | - | 2.25 |
| NS1 | 3009 | C | T | - | 1.07 | NS5 | 8604 | C | T | - | 2.69 |
| NS1 | 3012 | T | C | - | 1.75 | NS5 | 8634 | T | C | - | 16.67 |
| NS1 | 3152 | C | CA | Asn246fs | 4.23 | NS5 | 8658 | C | T | - | 11.76 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 0.94 | NS5 | 8891 | T | TA | His442fs | 1.25 |
| NS2A | 3657 | T | G | - | 5.80 | NS5 | 8926 | A | AC | Asn453fs | 2.07 |
| NS2A | 3678 | T | C | - | 6.36 | NS5 | 9309 | A | G | - | 10.81 |
| NS2A | 3821 | T | C | Ile115Thr | 4.81 | NS5 | 9512 | T | C | Val648Ala | 4.93 |
| NS2A | 3866 | G | C | Gly130Ala | 0.96 | NS5 | 9576 | C | T | - | 1.13 |
| NS2A | 3928 | G | C | Ala151Pro | 1.52 | NS5 | 9693 | T | C | - | 4.53 |
| NS2A | 4034 | G | A | Arg186Gln | 1.38 | NS5 | 9705 | T | TC | Phe713fs | 1.08 |
| NS2A | 4042 | A | G | Thr189Ala | 46.75 | NS5 | 9714 | G | C | Glu715Asp | 0.92 |
| NS2A | 4069 | A | G | Ile198Val | 1.43 | NS5 | 9718 | G | C | Val717Leu | 0.91 |
| NS2A | 4110 | C | T | - | 1.77 | NS5 | 9726 | AG | A | Asp720fs | 0.74 |
| NS2A | 4119 | T | C | - | 5.47 | NS5 | 9738 | A | G | - | 6.22 |

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|---|---|---|---|---|---|---|---|---|---|---|
| NS2B | 4414 | T | C | - | 5.04 | NS5 | 9795 | A | G | - | 2.61 |
| NS3 | 4573 | C | T | - | 1.88 | NS5 | 9828 | T | C | - | 1.01 |
| NS3 | 4596 | T | C | - | 2.77 | NS5 | 9902 | C | G | Ala778Gly | 1.27 |
| NS3 | 4635 | C | T | - | 2.10 | NS5 | 9903 | C | T | - | 1.16 |
| NS3 | 4707 | G | A | - | 3.09 | NS5 | 9942 | T | C | - | 3.38 |
| NS3 | 4878 | C | T | - | 0.73 | NS5 | 10212 | T | C | - | 4.43 |
| NS3 | 5026 | G | GA | Ser171fs | 5.69 | 3UTR | 10389 | CA | C | - | 9.90 |
| NS3 | 5120 | CA | C | Lys201fs | 0.54 | 3UTR | 10612 | C | CA | - | 4.48 |

| **191** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WS | | | | | | NS3 | 4944 | C | T | - | 39.62 |
| Secondary | | | | | | NS3 | 4971 | T | C | - | 27.97 |
| 3.78E+03 | | | | | | NS3 | 5004 | C | T | - | 37.10 |
| 0 | | | | | | NS3 | 5033 | G | A | Ser171Asn | 3.31 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5070 | C | T | - | 38.89 |
| C | 258 | T | C | - | 30.94 | NS3 | 5118 | G | A | - | 29.17 |
| C | 315 | G | A | - | 38.52 | NS3 | 5190 | C | T | - | 8.00 |
| C | 327 | T | C | - | 24.58 | NS3 | 5235 | T | C | - | 26.67 |
| C | 342 | A | G | - | 28.87 | NS3 | 5277 | C | T | - | 41.07 |
| C | 363 | A | G | - | 17.58 | NS3 | 5304 | T | C | - | 12.50 |
| C | 384 | C | T | - | 41.56 | NS3 | 5394 | A | G | - | 18.67 |
| C | 387 | G | A | - | 38.27 | NS3 | 5478 | G | A | - | 18.57 |
| C | 406 | G | T | Val104Leu | 13.33 | NS3 | 5502 | G | A | - | 24.29 |
| C | 411 | T | C | - | 23.58 | NS3 | 5613 | T | C | - | 41.18 |
| prM/M | 445 | T | C | - | 40.40 | NS3 | 5649 | G | A | - | 8.00 |
| prM/M | 504 | T | C | - | 24.53 | NS3 | 5667 | G | A | - | 12.50 |
| prM/M | 508 | C | T | - | 32.38 | NS3 | 5682 | G | A | - | 26.09 |
| prM/M | 567 | A | G | - | 27.36 | NS3 | 5708 | A | G | Lys396Arg | 13.79 |
| prM/M | 651 | G | A | - | 41.38 | NS3 | 5730 | T | C | - | 31.58 |
| prM/M | 732 | T | C | - | 39.53 | NS3 | 5796 | T | C | - | 13.33 |
| prM/M | 798 | C | T | - | 38.61 | NS3 | 5802 | T | C | - | 32.73 |
| prM/M | 820 | T | C | - | 24.37 | NS3 | 5823 | A | G | - | 8.20 |
| prM/M | 834 | T | C | - | 46.72 | NS3 | 5835 | A | G | - | 8.93 |
| prM/M | 837 | C | T | - | 40.83 | NS3 | 5844 | G | A | - | 11.32 |
| prM/M | 864 | C | T | - | 49.56 | NS3 | 5979 | A | C | - | 12.24 |
| prM/M | 873 | A | C | - | 44.19 | NS3 | 6009 | T | C | - | 37.78 |
| prM/M | 894 | C | A | - | 43.24 | NS3 | 6069 | G | T | - | 19.05 |
| prM/M | 921 | C | T | - | 22.81 | NS3 | 6094 | T | C | - | 12.24 |
| E | 1002 | C | T | - | 16.67 | NS3 | 6099 | A | G | - | 10.20 |
| E | 1005 | A | C | - | 41.28 | NS3 | 6157 | T | C | - | 16.67 |
| E | 1040 | C | CA | Asn37fs | 3.54 | NS3 | 6192 | C | T | - | 26.56 |
| E | 1102 | C | T | - | 27.94 | NS3 | 6198 | C | T | - | 7.04 |
| E | 1293 | T | C | - | 28.41 | NS3 | 6220 | A | G | Ile567Val | 31.88 |
| E | 1302 | A | G | - | 26.74 | NS3 | 6222 | T | C | - | 30.00 |
| E | 1353 | C | T | - | 36.70 | NS3 | 6292 | T | C | - | 30.43 |
| E | 1398 | G | C | Glu154Asp | 39.44 | NS3 | 6300 | C | T | - | 26.74 |
| E | 1473 | C | T | - | 39.39 | NS3 | 6324 | T | C | - | 11.63 |
| E | 1479 | C | T | - | 23.44 | NS3 | 6330 | C | T | - | 35.77 |
| E | 1641 | G | A | - | 35.37 | NS4A | 6378 | T | C | - | 28.93 |
| E | 1650 | C | T | - | 45.31 | NS4A | 6379 | T | C | - | 28.69 |
| E | 1701 | T | C | - | 11.70 | NS4A | 6462 | T | C | - | 24.74 |
| E | 1710 | G | T | - | 12.94 | NS4A | 6471 | T | C | - | 31.13 |
| E | 1842 | T | C | - | 27.08 | NS4A | 6496 | T | C | Tyr41His | 21.43 |
| E | 1863 | G | A | - | 18.75 | NS4A | 6500 | C | A | Thr42Asn | 18.95 |
| E | 1925 | G | A | Gly330Asp | 47.83 | NS4A | 6528 | T | C | - | 48.63 |
| E | 1944 | T | C | - | 39.34 | NS4A | 6547 | C | T | - | 22.75 |
| E | 1986 | C | T | - | 26.51 | NS4A | 6556 | C | T | - | 23.70 |
| E | 1995 | A | G | - | 40.24 | NS4A | 6562 | A | G | Thr63Ala | 48.17 |
| E | 2023 | A | G | Ser363Gly | 37.50 | NS4A | 6564 | A | G | - | 46.88 |

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|---|---|---|---|---|---|
| E | 2024 | G | C | | 40.28 |
| E | 2049 | T | C | - | 11.67 |
| E | 2055 | C | T | - | 43.08 |
| E | 2064 | C | T | - | 9.09 |
| E | 2073 | C | T | - | 48.28 |
| E | 2105 | A | G | Asn390Ser | 37.50 |
| E | 2210 | T | A | Leu425* | 3.92 |
| E | 2244 | C | T | - | 24.60 |
| E | 2276 | C | T | Ala447Val | 27.69 |
| E | 2289 | C | T | - | 24.54 |
| E | 2307 | C | T | - | 29.09 |
| E | 2319 | C | A | - | 2.87 |
| E | 2328 | A | T | - | 33.71 |
| E | 2355 | C | T | - | 33.14 |
| E | 2397 | G | A | - | 11.93 |
| NS1 | 2451 | T | C | - | 49.10 |
| NS1 | 2484 | T | C | - | 32.86 |
| NS1 | 2511 | A | G | - | 17.20 |
| NS1 | 2589 | A | G | - | 41.56 |
| NS1 | 2640 | A | G | - | 18.25 |
| NS1 | 2739 | T | C | - | 25.00 |
| NS1 | 2781 | G | A | - | 45.56 |
| NS1 | 2878 | C | A | Leu153Met | 34.86 |
| NS1 | 2898 | T | C | - | 36.54 |
| NS1 | 2952 | A | G | - | 20.95 |
| NS1 | 2961 | C | T | - | 36.00 |
| NS1 | 2976 | A | G | - | 45.24 |
| NS1 | 2985 | T | A | - | 3.23 |
| NS1 | 2991 | C | T | - | 25.83 |
| NS1 | 3042 | T | C | - | 18.02 |
| NS1 | 3114 | C | A | - | 21.54 |
| NS1 | 3162 | T | A | Phe247Leu | 6.45 |
| NS1 | 3186 | C | T | - | 15.12 |
| NS1 | 3234 | T | C | - | 34.13 |
| NS1 | 3330 | C | T | - | 6.75 |
| NS1 | 3402 | T | C | - | 38.35 |
| NS2A | 3489 | G | A | - | 43.40 |
| NS2A | 3624 | T | C | - | 48.65 |
| NS2A | 3629 | G | A | Arg51Lys | 47.79 |
| NS2A | 3664 | A | G | Thr63Ala | 18.55 |
| NS2A | 3753 | C | T | - | 38.85 |
| NS2A | 3768 | C | T | - | 42.40 |
| NS2A | 3801 | C | T | - | 19.85 |
| NS2A | 3864 | G | A | - | 43.88 |
| NS2A | 3875 | C | T | Ala133Val | 50.00 |
| NS2A | 3876 | C | T | - | 30.37 |
| NS2A | 3879 | C | A | - | 46.95 |
| NS2A | 3906 | C | T | - | 15.08 |
| NS2A | 3910 | T | C | - | 14.05 |
| NS2A | 3921 | T | C | - | 40.79 |
| NS2A | 4044 | G | A | - | 15.38 |
| NS2A | 4063 | T | C | - | 18.18 |
| NS2A | 4110 | T | C | - | 4.70 |
| NS2B | 4206 | T | C | - | 9.36 |
| NS2B | 4249 | G | A | Val40Ile | 14.39 |
| NS2B | 4254 | T | C | - | 19.71 |
| NS2B | 4257 | C | T | - | 12.50 |
| NS2B | 4278 | C | T | - | 44.09 |
| NS2B | 4329 | A | G | - | 44.71 |
| NS2B | 4338 | A | G | - | 42.17 |
| NS4A | 6585 | A | G | - | 48.59 |
| NS4A | 6606 | T | C | - | 13.40 |
| NS4A | 6633 | T | C | - | 26.63 |
| NS4A | 6652 | A | G | Ile93Val | 16.05 |
| NS4A | 6654 | T | C | - | 25.16 |
| NS4A | 6681 | A | G | - | 18.66 |
| NS4B | 6852 | C | T | - | 29.46 |
| NS4B | 6864 | C | T | - | 19.67 |
| NS4B | 6871 | G | A | Gly16Arg | 19.33 |
| NS4B | 6879 | T | C | - | 39.55 |
| NS4B | 6906 | C | T | - | 23.40 |
| NS4B | 6948 | T | C | - | 45.00 |
| NS4B | 6966 | T | C | - | 25.30 |
| NS4B | 6984 | A | G | - | 27.55 |
| NS4B | 7008 | G | A | - | 44.33 |
| NS4B | 7029 | T | A | - | 27.87 |
| NS4B | 7038 | A | G | - | 39.53 |
| NS4B | 7041 | C | T | - | 49.43 |
| NS4B | 7059 | T | C | - | 7.30 |
| NS4B | 7062 | A | G | - | 49.40 |
| NS4B | 7137 | T | C | - | 12.96 |
| NS4B | 7248 | C | T | - | 34.15 |
| NS4B | 7314 | A | G | - | 44.44 |
| NS4B | 7383 | T | C | - | 45.90 |
| NS4B | 7428 | C | T | - | 36.25 |
| NS4B | 7506 | G | A | - | 34.29 |
| NS4B | 7518 | C | T | - | 26.23 |
| NS4B | 7542 | C | T | - | 15.09 |
| NS5 | 7582 | G | A | Val5Ile | 22.86 |
| NS5 | 7656 | G | A | - | 43.04 |
| NS5 | 7716 | A | G | - | 33.96 |
| NS5 | 7758 | A | G | - | 47.22 |
| NS5 | 7767 | C | T | - | 42.42 |
| NS5 | 7797 | A | G | - | 47.83 |
| NS5 | 7812 | T | C | - | 28.13 |
| NS5 | 7911 | C | T | - | 36.11 |
| NS5 | 7941 | G | T | - | 28.86 |
| NS5 | 7999 | C | T | - | 43.56 |
| NS5 | 8028 | T | C | - | 22.30 |
| NS5 | 8103 | T | C | - | 21.37 |
| NS5 | 8127 | T | C | - | 14.50 |
| NS5 | 8184 | A | G | - | 41.11 |
| NS5 | 8241 | T | C | - | 40.40 |
| NS5 | 8284 | T | C | - | 43.27 |
| NS5 | 8289 | T | C | - | 29.13 |
| NS5 | 8544 | C | T | - | 41.33 |
| NS5 | 8562 | T | C | - | 21.69 |
| NS5 | 8569 | G | A | Val334Ile | 15.58 |
| NS5 | 8574 | C | T | - | 19.28 |
| NS5 | 8595 | A | G | - | 25.93 |
| NS5 | 8628 | C | T | - | 38.10 |
| NS5 | 8640 | G | A | - | 22.22 |
| NS5 | 8722 | C | T | - | 17.39 |
| NS5 | 8841 | G | A | - | 9.46 |
| NS5 | 8844 | T | C | - | 9.21 |
| NS5 | 9132 | G | A | - | 27.27 |
| NS5 | 9390 | C | T | - | 38.46 |
| NS5 | 9501 | G | C | Gln644His | 47.06 |
| NS5 | 9605 | C | T | Thr679Ile | 22.22 |
| NS5 | 9628 | G | A | Val687Ile | 22.35 |

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|---|---|---|---|---|---|
| NS2B | 4341 | C | T | - | 33.71 |
| NS2B | 4344 | C | T | - | 36.73 |
| NS2B | 4350 | C | T | - | 14.43 |
| NS2B | 4414 | C | T | - | 22.22 |
| NS2B | 4491 | A | G | - | 37.36 |
| NS2B | 4509 | G | T | - | 39.39 |
| NS3 | 4524 | T | C | - | 23.53 |
| NS3 | 4587 | C | T | - | 7.79 |
| NS3 | 4590 | T | C | - | 25.35 |
| NS3 | 4596 | C | T | - | 47.14 |
| NS3 | 4626 | G | A | - | 22.58 |
| NS3 | 4662 | T | C | - | 5.80 |
| NS3 | 4731 | A | G | - | 6.25 |
| NS3 | 4739 | A | G | Lys73Arg | 5.77 |
| NS3 | 4782 | A | G | - | 34.43 |
| NS3 | 4806 | C | T | - | 25.00 |
| NS3 | 4812 | C | T | - | 22.22 |
| NS3 | 4830 | A | G | - | 26.67 |
| NS3 | 4854 | G | A | - | 26.42 |
| NS3 | 4875 | C | T | - | 25.44 |
| NS3 | 4905 | G | A | - | 37.40 |
| NS5 | 9657 | T | C | - | 26.19 |
| NS5 | 9660 | A | T | - | 12.79 |
| NS5 | 9705 | T | TC | Phe713fs | 2.68 |
| NS5 | 9725 | A | T | Lys719Ile | 12.75 |
| NS5 | 9768 | A | G | - | 13.89 |
| NS5 | 9789 | T | C | - | 15.89 |
| NS5 | 9840 | T | C | - | 21.60 |
| NS5 | 9849 | A | G | - | 21.58 |
| NS5 | 9902 | C | G | Ala778Gly | 3.55 |
| NS5 | 9942 | T | C | - | 20.73 |
| NS5 | 10083 | C | T | - | 37.93 |
| NS5 | 10140 | T | C | - | 25.00 |
| NS5 | 10252 | G | A | Glu895Lys | 38.10 |
| 3UTR | 10280 | G | A | - | 31.58 |
| 3UTR | 10296 | A | G | - | 43.48 |
| 3UTR | 10389 | C | CA | - | 2.97 |
| 3UTR | 10389 | C | CAA | - | 28.71 |
| 3UTR | 10389 | C | T | - | 17.31 |
| 3UTR | 10407 | T | C | - | 31.73 |
| 3UTR | 10443 | T | TA | - | 2.38 |
| 3UTR | 10611 | C | CAAA | - | 8.70 |

| **192** | | | | | |
|---|---|---|---|---|---|
| WS | | | | | |
| Secondary | | | | | |
| 7.33E+03 | | | | | |
| 2 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 70 | G | GT | - | 2.01 |
| C | 125 | G | T | Ser10Ile | 34.91 |
| C | 168 | A | G | - | 2.35 |
| C | 181 | C | T | - | 2.40 |
| C | 213 | A | G | - | 1.81 |
| C | 219 | C | A | - | 1.86 |
| C | 228 | A | G | - | 1.75 |
| C | 284 | C | T | Ala63Val | 1.65 |
| C | 294 | A | G | - | 2.03 |
| C | 315 | G | A | - | 2.81 |
| C | 318 | A | G | - | 2.20 |
| C | 324 | A | G | - | 3.00 |
| C | 327 | T | C | - | 9.73 |
| C | 336 | T | C | - | 2.87 |
| C | 345 | G | C | - | 2.19 |
| C | 375 | C | T | - | 2.51 |
| C | 384 | C | T | - | 4.03 |
| C | 431 | C | T | Ala112Val | 9.77 |
| C | 432 | G | A | - | 2.27 |
| prM/M | 508 | C | T | - | 3.21 |
| prM/M | 513 | C | T | - | 1.94 |
| prM/M | 523 | G | A | Asp29Asn | 8.92 |
| prM/M | 525 | T | C | - | 10.23 |
| prM/M | 531 | T | G | - | 2.65 |
| prM/M | 531 | T | C | - | 8.85 |
| prM/M | 567 | G | A | - | 3.54 |
| prM/M | 579 | T | C | - | 2.41 |
| prM/M | 583 | A | G | Ile49Val | 2.06 |
| prM/M | 588 | G | T | - | 1.95 |
| prM/M | 591 | C | T | - | 1.95 |
| prM/M | 594 | G | C | Lys52Asn | 1.99 |
| NS2B | 4344 | C | T | - | 6.21 |
| NS2B | 4350 | T | C | - | 4.52 |
| NS2B | 4386 | G | A | - | 4.44 |
| NS2B | 4401 | G | A | - | 4.72 |
| NS2B | 4434 | G | A | - | 9.28 |
| NS2B | 4441 | T | C | - | 5.98 |
| NS2B | 4449 | C | A | - | 4.20 |
| NS2B | 4456 | G | C | Val109Leu | 3.68 |
| NS2B | 4458 | C | T | - | 4.11 |
| NS2B | 4464 | A | C | - | 4.29 |
| NS2B | 4470 | G | A | - | 3.80 |
| NS2B | 4479 | T | C | - | 3.77 |
| NS2B | 4491 | A | G | - | 2.99 |
| NS2B | 4509 | G | T | - | 2.99 |
| NS3 | 4530 | A | G | - | 3.11 |
| NS3 | 4539 | C | T | - | 5.04 |
| NS3 | 4542 | C | T | - | 4.96 |
| NS3 | 4557 | A | C | - | 6.92 |
| NS3 | 4566 | A | G | - | 5.88 |
| NS3 | 4596 | C | T | - | 6.60 |
| NS3 | 4620 | T | C | - | 4.12 |
| NS3 | 4623 | T | C | - | 5.15 |
| NS3 | 4629 | T | C | - | 4.60 |
| NS3 | 4635 | C | T | - | 4.82 |
| NS3 | 4641 | T | C | - | 4.94 |
| NS3 | 4662 | T | C | - | 5.05 |
| NS3 | 4746 | T | C | - | 6.04 |
| NS3 | 4806 | T | C | - | 9.62 |
| NS3 | 4829 | G | GA | Asn105fs | 1.61 |
| NS3 | 4854 | A | G | - | 14.57 |
| NS3 | 4946 | G | A | Arg142Lys | 1.95 |
| NS3 | 4959 | T | C | - | 4.36 |
| NS3 | 4971 | T | C | - | 7.10 |
| NS3 | 5070 | C | T | - | 3.03 |
| NS3 | 5082 | A | G | - | 9.94 |
| NS3 | 5121 | A | G | - | 2.63 |
| NS3 | 5139 | C | A | - | 2.49 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| prM/M | 600 | C | T | - | 2.35 | NS3 | 5145 | T | C | - | 3.09 |
| prM/M | 603 | C | T | - | 2.51 | NS3 | 5151 | A | G | - | 3.67 |
| prM/M | 612 | A | G | - | 2.94 | NS3 | 5235 | C | T | - | 17.12 |
| prM/M | 633 | T | C | - | 2.51 | NS3 | 5277 | C | T | - | 6.77 |
| prM/M | 651 | G | A | - | 2.21 | NS3 | 5283 | A | G | - | 5.88 |
| prM/M | 703 | G | GA | Arg91fs | 3.67 | NS3 | 5286 | G | A | - | 2.96 |
| prM/M | 717 | G | A | - | 19.64 | NS3 | 5295 | C | T | - | 2.30 |
| prM/M | 723 | T | C | - | 19.09 | NS3 | 5296 | C | T | - | 2.47 |
| prM/M | 792 | G | A | - | 4.09 | NS3 | 5319 | T | C | - | 5.78 |
| prM/M | 798 | T | C | - | 3.45 | NS3 | 5340 | C | T | - | 4.86 |
| prM/M | 837 | T | C | - | 2.38 | NS3 | 5433 | A | T | - | 9.09 |
| prM/M | 852 | G | A | - | 3.76 | NS3 | 5436 | G | A | - | 10.64 |
| prM/M | 876 | G | A | - | 1.79 | NS3 | 5544 | G | A | - | 2.03 |
| prM/M | 880 | C | T | His148Tyr | 1.75 | NS3 | 5610 | A | G | - | 4.69 |
| prM/M | 882 | C | T | - | 1.67 | NS3 | 5649 | G | A | - | 6.54 |
| prM/M | 891 | G | A | - | 2.56 | NS3 | 5667 | A | G | - | 8.18 |
| prM/M | 893 | C | T | Ala152Val | 2.62 | NS3 | 5751 | C | T | - | 14.56 |
| prM/M | 894 | C | A | - | 2.05 | NS3 | 5754 | G | A | - | 14.38 |
| prM/M | 921 | T | C | - | 20.62 | NS3 | 5802 | C | T | - | 3.45 |
| E | 954 | A | C | - | 1.62 | NS3 | 5823 | A | G | - | 3.72 |
| E | 960 | T | C | - | 4.49 | NS3 | 5832 | C | T | - | 2.27 |
| E | 969 | C | T | - | 3.29 | NS3 | 5859 | T | C | - | 7.82 |
| E | 972 | A | G | - | 3.26 | NS3 | 5868 | G | A | - | 2.48 |
| E | 1005 | C | A | - | 5.61 | NS3 | 6033 | C | T | - | 3.03 |
| E | 1008 | T | C | - | 3.82 | NS3 | 6061 | G | GA | Val516fs | 2.07 |
| E | 1023 | T | C | - | 15.36 | NS3 | 6157 | T | C | - | 9.14 |
| E | 1032 | G | A | - | 3.63 | NS3 | 6159 | G | A | - | 5.65 |
| E | 1040 | C | CA | Asn37fs | 1.08 | NS3 | 6167 | A | G | Lys549Arg | 4.19 |
| E | 1062 | T | C | - | 2.95 | NS3 | 6177 | T | C | - | 4.57 |
| E | 1293 | T | C | - | 3.80 | NS3 | 6186 | C | T | - | 3.81 |
| E | 1413 | A | C | - | 12.63 | NS3 | 6192 | C | T | - | 3.11 |
| E | 1473 | C | T | - | 11.02 | NS3 | 6203 | A | G | Lys588Arg | 2.88 |
| E | 1545 | C | T | - | 14.84 | NS3 | 6220 | A | G | Ile567Val | 4.15 |
| E | 1590 | A | G | - | 5.26 | NS3 | 6222 | T | C | - | 4.15 |
| E | 1599 | A | G | - | 11.85 | NS3 | 6279 | G | A | - | 2.92 |
| E | 1626 | T | C | - | 10.17 | NS3 | 6294 | A | G | - | 3.96 |
| E | 1842 | C | T | - | 18.57 | NS3 | 6303 | A | G | - | 3.45 |
| E | 1858 | A | G | Ile308Val | 5.38 | NS3 | 6307 | T | C | - | 3.47 |
| E | 1860 | C | T | - | 5.47 | NS3 | 6312 | T | C | - | 3.79 |
| E | 1863 | G | A | - | 5.51 | NS3 | 6330 | T | C | - | 13.41 |
| E | 1944 | T | C | - | 5.83 | NS3 | 6333 | A | G | - | 2.60 |
| E | 1959 | T | C | - | 5.69 | NS3 | 6339 | A | G | - | 2.64 |
| E | 1972 | C | T | His346Tyr | 5.80 | NS3 | 6342 | A | C | - | 2.65 |
| E | 1995 | A | G | - | 1.85 | NS3 | 6354 | G | A | - | 2.77 |
| E | 2007 | T | C | - | 9.09 | NS4A | 6378 | T | C | - | 4.55 |
| E | 2023 | A | G | Ser363Gly | 3.76 | NS4A | 6379 | T | C | - | 4.18 |
| E | 2024 | G | C | | 3.68 | NS4A | 6390 | C | T | - | 2.51 |
| E | 2049 | C | T | - | 10.20 | NS4A | 6471 | C | T | - | 14.25 |
| E | 2064 | T | C | - | 15.60 | NS4A | 6512 | G | A | Ser46Asn | 14.21 |
| E | 2067 | C | T | - | 6.16 | NS4A | 6532 | G | GC | Glu53fs | 0.91 |
| E | 2073 | C | T | - | 7.81 | NS4A | 6585 | G | A | - | 7.83 |
| E | 2163 | A | G | - | 5.94 | NS4A | 6615 | G | A | - | 2.61 |
| E | 2223 | T | C | - | 5.83 | NS4A | 6633 | T | C | - | 4.42 |
| E | 2241 | T | C | - | 2.69 | NS4A | 6648 | T | C | - | 2.68 |
| E | 2247 | T | C | - | 5.45 | NS4A | 6651 | C | T | - | 3.04 |
| E | 2256 | C | T | - | 4.17 | NS4A | 6732 | C | T | - | 2.63 |
| E | 2298 | T | C | - | 3.29 | NS4A | 6777 | G | A | - | 6.44 |
| E | 2319 | C | A | - | 3.10 | NS4A | 6780 | C | T | - | 6.49 |
| E | 2320 | G | A | Val462Ile | 13.24 | NS4B | 6837 | T | G | - | 3.33 |
| E | 2328 | T | A | - | 16.01 | NS4B | 6840 | C | T | - | 3.33 |

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|---|---|---|---|---|---|
| E | 2349 | T | C | - | 3.53 |
| E | 2367 | G | A | - | 1.95 |
| E | 2376 | A | G | - | 10.10 |
| NS1 | 2511 | G | A | - | 3.73 |
| NS1 | 2911 | T | A | Ser164Thr | 7.57 |
| NS1 | 2961 | C | T | - | 1.72 |
| NS1 | 2976 | A | G | - | 3.05 |
| NS1 | 2989 | G | A | Asp190Asn | 2.42 |
| NS1 | 2991 | T | C | - | 4.71 |
| NS1 | 3009 | C | T | - | 3.72 |
| NS1 | 3081 | T | A | - | 4.27 |
| NS1 | 3114 | A | C | - | 7.65 |
| NS1 | 3129 | G | A | - | 8.19 |
| NS1 | 3130 | T | C | - | 2.81 |
| NS1 | 3152 | C | CA | Asn246fs | 2.52 |
| NS1 | 3165 | C | T | - | 5.30 |
| NS1 | 3168 | G | A | - | 4.85 |
| NS1 | 3186 | C | T | - | 4.27 |
| NS1 | 3258 | C | T | - | 3.87 |
| NS1 | 3261 | C | T | - | 3.93 |
| NS1 | 3270 | T | C | - | 3.30 |
| NS1 | 3289 | G | A | Asp290Asn | 2.95 |
| NS1 | 3313 | T | C | - | 2.60 |
| NS1 | 3324 | C | T | - | 1.64 |
| NS1 | 3327 | T | C | - | 1.93 |
| NS1 | 3330 | C | T | - | 1.87 |
| NS1 | 3339 | G | A | - | 1.72 |
| NS1 | 3366 | C | T | - | 1.74 |
| NS1 | 3438 | A | G | - | 5.15 |
| NS1 | 3447 | A | G | - | 5.42 |
| NS1 | 3474 | A | G | - | 7.32 |
| NS2A | 3552 | C | T | - | 21.74 |
| NS2A | 3597 | C | T | - | 11.45 |
| NS2A | 3600 | G | T | - | 5.30 |
| NS2A | 3604 | T | C | - | 11.27 |
| NS2A | 3624 | T | C | - | 5.49 |
| NS2A | 3629 | G | A | Arg51Lys | 6.59 |
| NS2A | 3663 | T | C | - | 17.01 |
| NS2A | 3768 | C | T | - | 2.38 |
| NS2A | 3844 | T | C | - | 17.73 |
| NS2A | 3858 | A | G | - | 1.62 |
| NS2A | 3864 | G | A | - | 3.93 |
| NS2A | 3874 | G | A | Val133Ile | 2.15 |
| NS2A | 3875 | C | T | Ala133Val | 3.68 |
| NS2A | 3876 | T | C | - | 16.59 |
| NS2A | 3879 | C | A | - | 1.63 |
| NS2A | 3900 | A | G | - | 1.81 |
| NS2A | 3903 | A | G | - | 1.79 |
| NS2A | 3906 | C | T | - | 1.61 |
| NS2A | 3912 | G | A | - | 1.92 |
| NS2A | 3935 | C | T | Ser153Leu | 1.83 |
| NS2A | 3936 | G | A | - | 1.51 |
| NS2A | 3942 | C | T | - | 1.68 |
| NS2A | 3966 | C | T | - | 1.25 |
| NS2A | 4011 | T | C | - | 4.03 |
| NS2A | 4034 | A | G | Gln186Arg | 1.63 |
| NS2A | 4063 | C | T | - | 23.20 |
| NS2A | 4083 | C | T | - | 4.86 |
| NS2A | 4098 | T | C | - | 3.19 |
| NS2A | 4107 | T | C | - | 3.20 |
| NS4B | 6844 | G | GA | Thr9fs | 1.10 |
| NS4B | 6852 | C | G | - | 2.72 |
| NS4B | 6864 | T | C | - | 15.11 |
| NS4B | 6871 | A | G | Arg16Gly | 16.10 |
| NS4B | 6884 | T | C | Ile20Thr | 15.55 |
| NS4B | 6943 | T | C | - | 17.49 |
| NS4B | 6957 | T | C | - | 14.04 |
| NS4B | 6967 | G | A | Val48Ile | 1.85 |
| NS4B | 7008 | G | A | - | 16.51 |
| NS4B | 7014 | C | G | - | 1.47 |
| NS4B | 7018 | C | T | - | 15.21 |
| NS4B | 7032 | T | C | - | 1.98 |
| NS4B | 7038 | A | G | - | 2.76 |
| NS4B | 7041 | T | C | - | 6.88 |
| NS4B | 7083 | G | A | - | 1.86 |
| NS4B | 7104 | C | T | - | 1.57 |
| NS4B | 7137 | T | C | - | 4.50 |
| NS4B | 7152 | A | T | - | 3.26 |
| NS4B | 7165 | T | C | - | 3.75 |
| NS4B | 7186 | A | G | ile121Val | 10.73 |
| NS4B | 7248 | C | T | - | 2.15 |
| NS4B | 7318 | G | GA | Gln167fs | 1.46 |
| NS4B | 7410 | C | T | - | 11.30 |
| NS4B | 7428 | C | T | - | 5.62 |
| NS4B | 7449 | C | T | - | 15.38 |
| NS4B | 7518 | C | T | - | 6.90 |
| NS5 | 7777 | A | C | Met70Leu | 10.00 |
| NS5 | 7788 | G | A | - | 10.34 |
| NS5 | 7794 | G | A | - | 22.03 |
| NS5 | 7911 | C | T | - | 2.83 |
| NS5 | 7947 | G | T | - | 2.03 |
| NS5 | 7959 | C | T | - | 3.96 |
| NS5 | 7971 | C | T | - | 1.76 |
| NS5 | 8083 | C | T | - | 1.66 |
| NS5 | 8089 | G | A | Asp174Asn | 12.14 |
| NS5 | 8103 | C | T | - | 13.71 |
| NS5 | 8184 | A | G | - | 4.71 |
| NS5 | 8203 | C | A | - | 2.02 |
| NS5 | 8208 | C | T | - | 2.62 |
| NS5 | 8214 | G | A | - | 1.96 |
| NS5 | 8220 | A | G | - | 2.17 |
| NS5 | 8226 | C | T | - | 2.57 |
| NS5 | 8235 | T | C | - | 3.95 |
| NS5 | 8242 | A | T | Thr225Ser | 5.22 |
| NS5 | 8284 | T | C | - | 2.74 |
| NS5 | 8289 | T | C | - | 3.85 |
| NS5 | 8306 | A | G | Lys246Arg | 4.79 |
| NS5 | 8496 | T | C | - | 8.42 |
| NS5 | 8508 | A | G | - | 8.93 |
| NS5 | 8526 | T | C | - | 8.67 |
| NS5 | 8535 | C | T | - | 7.59 |
| NS5 | 8541 | G | A | - | 7.38 |
| NS5 | 8544 | C | T | - | 10.74 |
| NS5 | 8572 | G | A | Val335Ile | 3.28 |
| NS5 | 8574 | T | C | - | 10.16 |
| NS5 | 8628 | C | T | - | 19.44 |
| NS5 | 8658 | T | C | - | 16.67 |
| NS5 | 8823 | G | A | - | 5.82 |
| NS5 | 8891 | T | TA | His442fs | 1.81 |
| NS5 | 8926 | A | AC | Asn453fs | 2.88 |

| | | | | | |
|---|---|---|---|---|---|
| NS2A | 4113 | G | A | - | 2.09 |
| NS2A | 4121 | G | A | Ser215Asn | 2.39 |
| NS2B | 4134 | C | T | - | 1.93 |
| NS2B | 4149 | A | G | - | 2.10 |
| NS2B | 4164 | C | T | - | 2.95 |
| NS2B | 4197 | A | G | - | 2.32 |
| NS2B | 4206 | T | C | - | 3.83 |
| NS2B | 4212 | T | C | - | 2.86 |
| NS2B | 4221 | T | A | - | 3.65 |
| NS2B | 4225 | T | C | - | 3.83 |
| NS2B | 4242 | C | T | - | 5.67 |
| NS2B | 4248 | C | T | - | 4.78 |
| NS2B | 4251 | A | G | - | 5.04 |
| NS2B | 4270 | C | A | - | 4.76 |
| NS2B | 4278 | C | T | - | 8.38 |
| NS2B | 4305 | T | C | - | 3.33 |
| NS2B | 4310 | A | G | Lys60Arg | 3.14 |
| NS2B | 4323 | G | A | - | 2.23 |
| NS2B | 4329 | A | G | - | 5.43 |
| NS2B | 4338 | A | G | - | 3.39 |
| NS2B | 4341 | C | T | - | 4.79 |
| NS5 | 8979 | T | C | - | 7.34 |
| NS5 | 9012 | A | G | - | 6.92 |
| NS5 | 9066 | C | T | - | 5.34 |
| NS5 | 9399 | T | C | - | 8.43 |
| NS5 | 9501 | G | C | Gln644His | 3.35 |
| NS5 | 9628 | A | G | Ile687Val | 13.55 |
| NS5 | 9705 | T | TC | Phe713fs | 1.69 |
| NS5 | 9795 | G | A | - | 2.84 |
| NS5 | 9825 | T | C | - | 4.21 |
| NS5 | 9840 | C | T | - | 18.18 |
| NS5 | 9849 | G | A | - | 17.57 |
| NS5 | 9888 | A | G | - | 12.27 |
| NS5 | 9900 | T | C | - | 2.65 |
| NS5 | 9903 | C | T | - | 2.54 |
| NS5 | 9921 | G | A | - | 2.69 |
| NS5 | 10075 | G | A | Val836Ile | 19.44 |
| NS5 | 10089 | G | A | - | 1.79 |
| NS5 | 10202 | G | A | Gly878Glu | 7.50 |
| NS5 | 10252 | G | A | Glu895Lys | 23.08 |
| 3UTR | 10389 | C | CA | - | 0.86 |
| 3UTR | 10443 | T | TA | - | 1.95 |

| **193** | | | | | |
|---|---|---|---|---|---|
| WS | | | | | |
| Primary | | | | | |
| 2.61E+04 | | | | | |
| 3 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 127 | A | T | Thr11Ser | 6.59 |
| C | 201 | C | T | - | 2.78 |
| C | 231 | A | G | - | 3.66 |
| C | 312 | C | CA | Ser75fs | 1.71 |
| C | 336 | C | T | - | 10.99 |
| C | 384 | T | C | - | 4.04 |
| C | 387 | A | G | - | 4.32 |
| C | 406 | G | A | Val104Met | 13.09 |
| C | 431 | C | T | Ala112Val | 3.28 |
| prM/M | 445 | C | T | - | 7.58 |
| prM/M | 450 | T | C | - | 14.84 |
| prM/M | 567 | A | G | - | 8.16 |
| prM/M | 651 | A | G | - | 7.04 |
| prM/M | 717 | G | A | - | 14.29 |
| prM/M | 798 | C | T | - | 4.55 |
| prM/M | 834 | C | T | - | 5.86 |
| prM/M | 837 | C | T | - | 5.79 |
| prM/M | 856 | T | C | - | 7.09 |
| prM/M | 912 | A | G | - | 23.98 |
| E | 954 | C | A | - | 14.22 |
| E | 969 | C | T | - | 3.07 |
| E | 1005 | A | C | - | 5.88 |
| E | 1023 | T | C | - | 5.96 |
| E | 1040 | C | CA | Asn37fs | 2.55 |
| E | 1094 | C | T | Pro53Leu | 4.96 |
| E | 1102 | T | C | - | 10.09 |
| E | 1214 | A | G | Lys93Arg | 4.40 |
| E | 1353 | C | T | - | 9.09 |
| E | 1473 | T | C | - | 7.82 |
| E | 1509 | C | T | - | 3.89 |
| E | 1590 | A | G | - | 27.35 |
| NS3 | 4662 | T | C | - | 11.82 |
| NS3 | 4695 | G | A | - | 7.14 |
| NS3 | 4707 | A | G | - | 29.41 |
| NS3 | 4830 | G | A | - | 4.17 |
| NS3 | 4905 | A | G | - | 5.38 |
| NS3 | 4944 | T | C | - | 6.20 |
| NS3 | 4959 | C | T | - | 4.58 |
| NS3 | 4974 | C | T | - | 14.57 |
| NS3 | 5026 | G | GA | Ser171fs | 6.01 |
| NS3 | 5070 | T | C | - | 2.89 |
| NS3 | 5181 | T | C | - | 24.66 |
| NS3 | 5182 | C | T | - | 2.63 |
| NS3 | 5190 | T | C | - | 13.20 |
| NS3 | 5277 | T | C | - | 7.29 |
| NS3 | 5304 | C | G | Cys261Trp | 3.31 |
| NS3 | 5304 | C | T | - | 12.40 |
| NS3 | 5319 | C | T | - | 19.74 |
| NS3 | 5478 | G | A | - | 4.05 |
| NS3 | 5637 | G | A | - | 21.79 |
| NS3 | 5730 | C | T | - | 11.70 |
| NS3 | 5802 | T | C | - | 12.00 |
| NS3 | 5835 | G | A | - | 21.25 |
| NS3 | 5844 | G | A | - | 7.69 |
| NS3 | 5847 | C | T | - | 5.26 |
| NS3 | 5915 | C | CA | Asn467fs | 6.98 |
| NS3 | 5979 | A | C | - | 6.03 |
| NS3 | 6009 | C | T | - | 2.84 |
| NS3 | 6069 | T | G | - | 7.00 |
| NS3 | 6094 | C | T | - | 7.96 |
| NS3 | 6099 | G | A | - | 8.55 |
| NS3 | 6198 | C | T | - | 3.87 |
| NS3 | 6222 | C | T | - | 7.97 |
| NS3 | 6294 | A | G | - | 3.74 |
| NS3 | 6300 | T | C | - | 15.18 |
| NS4A | 6378 | C | T | - | 6.32 |
| NS4A | 6379 | C | T | - | 5.81 |
| NS4A | 6462 | C | T | - | 9.04 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1641 | A | G | - | 4.66 | NS4A | 6471 | T | C | - | 4.19 |
| E | 1710 | G | T | - | 6.92 | NS4A | 6491 | G | A | Arg39Lys | 10.64 |
| E | 1860 | T | C | - | 12.50 | NS4A | 6496 | C | T | His41Tyr | 11.24 |
| E | 1863 | A | G | - | 7.95 | NS4A | 6500 | A | C | Asn42Thr | 9.13 |
| E | 1920 | G | T | - | 5.88 | NS4A | 6525 | G | A | - | 4.29 |
| E | 1995 | G | A | - | 4.59 | NS4A | 6528 | C | T | - | 7.59 |
| E | 2023 | G | A | Ala363Ser | 2.25 | NS4A | 6547 | T | C | - | 10.91 |
| E | 2024 | C | G | | 3.35 | NS4A | 6556 | T | C | - | 7.86 |
| E | 2055 | C | T | - | 4.17 | NS4A | 6562 | A | G | Thr63Ala | 7.39 |
| E | 2169 | A | G | - | 5.99 | NS4A | 6570 | A | G | - | 4.53 |
| E | 2220 | G | A | - | 7.35 | NS4A | 6585 | A | G | - | 4.25 |
| E | 2223 | C | T | - | 7.52 | NS4A | 6588 | C | T | - | 4.58 |
| E | 2244 | C | T | - | 2.38 | NS4A | 6591 | A | G | - | 4.94 |
| E | 2247 | C | T | - | 4.84 | NS4A | 6615 | G | A | - | 4.33 |
| E | 2256 | C | T | - | 3.45 | NS4A | 6633 | C | T | - | 3.35 |
| E | 2276 | C | T | Ala447Val | 6.93 | NS4A | 6654 | C | T | - | 8.33 |
| E | 2289 | T | C | - | 6.60 | NS4A | 6681 | G | A | - | 10.18 |
| E | 2307 | T | C | - | 5.07 | NS4A | 6711 | G | GT | Leu115fs | 3.51 |
| E | 2319 | A | C | - | 7.91 | NS4A | 6732 | C | T | - | 5.70 |
| E | 2349 | T | C | - | 2.09 | NS4A | 6777 | G | A | - | 4.24 |
| NS1 | 2434 | G | A | Val5Ile | 4.31 | NS4B | 6833 | T | TG | Leu6fs | 1.22 |
| NS1 | 2448 | G | A | - | 16.06 | NS4B | 6841 | T | C | - | 7.43 |
| NS1 | 2484 | C | T | - | 11.23 | NS4B | 6922 | C | T | Arg33Cys | 2.77 |
| NS1 | 2494 | G | A | Val25Met | 3.21 | NS4B | 6943 | C | T | - | 6.94 |
| NS1 | 2511 | A | G | - | 3.75 | NS4B | 6948 | C | T | - | 6.02 |
| NS1 | 2560 | CA | C | Gln47fs | 1.24 | NS4B | 6951 | C | A | - | 4.76 |
| NS1 | 2878 | A | C | Met153Leu | 7.14 | NS4B | 6982 | C | T | Arg53* | 1.59 |
| NS1 | 2898 | C | T | - | 5.59 | NS4B | 6990 | C | T | - | 1.97 |
| NS1 | 2952 | A | G | - | 4.29 | NS4B | 7008 | G | A | - | 4.92 |
| NS1 | 2961 | T | C | - | 7.74 | NS4B | 7038 | A | G | - | 6.89 |
| NS1 | 2976 | G | A | - | 8.55 | NS4B | 7041 | C | T | - | 7.79 |
| NS1 | 2989 | G | A | Asp190Asn | 3.95 | NS4B | 7059 | C | T | - | 18.86 |
| NS1 | 3009 | C | T | - | 2.31 | NS4B | 7062 | G | A | - | 10.60 |
| NS1 | 3075 | C | T | - | 9.02 | NS4B | 7083 | A | G | - | 23.26 |
| NS1 | 3152 | C | CA | Asn246fs | 3.88 | NS4B | 7092 | T | C | - | 21.14 |
| NS1 | 3234 | C | T | - | 6.70 | NS4B | 7104 | C | T | - | 1.82 |
| NS1 | 3330 | T | C | - | 15.96 | NS4B | 7137 | C | T | - | 14.81 |
| NS1 | 3342 | C | T | - | 4.30 | NS4B | 7143 | C | T | - | 5.58 |
| NS1 | 3402 | C | T | - | 9.01 | NS4B | 7146 | C | T | - | 5.11 |
| NS1 | 3414 | C | T | - | 6.19 | NS4B | 7165 | C | T | - | 11.84 |
| NS1 | 3432 | C | T | - | 8.33 | NS4B | 7314 | G | A | - | 4.30 |
| NS1 | 3438 | G | A | - | 8.79 | NS4B | 7356 | T | C | - | 4.80 |
| NS1 | 3447 | G | A | - | 10.00 | NS4B | 7371 | G | A | - | 27.06 |
| NS1 | 3474 | G | A | - | 7.32 | NS4B | 7383 | C | T | - | 4.13 |
| NS2A | 3489 | G | A | - | 19.64 | NS4B | 7428 | T | C | - | 6.51 |
| NS2A | 3552 | T | C | - | 3.61 | NS4B | 7518 | T | C | - | 4.58 |
| NS2A | 3595 | T | C | Phe40Leu | 4.11 | NS4B | 7551 | T | C | - | 15.53 |
| NS2A | 3597 | T | C | - | 4.19 | NS5 | 7656 | A | G | - | 14.29 |
| NS2A | 3624 | C | T | - | 5.43 | NS5 | 7758 | A | G | - | 9.43 |
| NS2A | 3629 | A | G | Lys51Arg | 3.93 | NS5 | 7776 | T | C | - | 12.77 |
| NS2A | 3750 | A | G | - | 2.90 | NS5 | 7848 | A | C | - | 5.31 |
| NS2A | 3753 | T | C | - | 5.07 | NS5 | 7911 | T | C | - | 5.39 |
| NS2A | 3768 | T | C | - | 4.54 | NS5 | 7959 | T | C | - | 4.41 |
| NS2A | 3801 | C | T | - | 11.72 | NS5 | 7995 | A | G | - | 3.81 |
| NS2A | 3864 | A | G | - | 6.52 | NS5 | 7996 | C | T | - | 20.07 |
| NS2A | 3866 | G | C | Gly130Ala | 1.42 | NS5 | 7999 | T | C | - | 11.75 |
| NS2A | 3875 | T | C | Val133Ala | 5.91 | NS5 | 8049 | G | A | - | 4.65 |
| NS2A | 3876 | C | T | - | 3.56 | NS5 | 8083 | T | C | - | 4.10 |
| NS2A | 3879 | A | C | - | 7.51 | NS5 | 8121 | C | T | - | 4.12 |
| NS2A | 3910 | T | C | - | 8.02 | NS5 | 8139 | C | T | - | 2.87 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2A | 3928 | G | C | Ala151Pro | 2.35 | NS5 | 8184 | G | A | - | 3.93 |
| NS2A | 4011 | C | T | - | 3.66 | NS5 | 8214 | G | A | - | 9.47 |
| NS2A | 4021 | C | T | - | 2.80 | NS5 | 8235 | T | C | - | 11.92 |
| NS2A | 4034 | G | A | Arg186Gln | 17.74 | NS5 | 8241 | C | T | - | 14.63 |
| NS2A | 4057 | C | T | - | 2.09 | NS5 | 8284 | C | T | - | 11.86 |
| NS2A | 4063 | T | C | - | 3.19 | NS5 | 8298 | T | C | - | 26.00 |
| NS2A | 4069 | G | A | Val198Ile | 28.08 | NS5 | 8310 | C | T | - | 12.50 |
| NS2A | 4083 | T | C | - | 3.99 | NS5 | 8364 | T | C | - | 7.14 |
| NS2A | 4098 | T | C | - | 2.19 | NS5 | 8469 | T | C | - | 5.08 |
| NS2A | 4110 | C | T | - | 17.57 | NS5 | 8544 | T | C | - | 10.58 |
| NS2A | 4121 | G | A | Ser215Asn | 2.39 | NS5 | 8619 | G | A | - | 16.13 |
| NS2B | 4134 | C | T | - | 3.33 | NS5 | 8658 | C | T | - | 10.20 |
| NS2B | 4164 | T | C | - | 8.87 | NS5 | 8803 | A | G | Ile412Val | 12.68 |
| NS2B | 4206 | C | T | - | 15.13 | NS5 | 8823 | A | G | - | 5.32 |
| NS2B | 4249 | G | A | Val40Ile | 7.84 | NS5 | 8926 | A | AC | Asn453fs | 2.61 |
| NS2B | 4251 | A | G | - | 4.49 | NS5 | 8967 | T | C | - | 9.17 |
| NS2B | 4257 | C | T | - | 7.11 | NS5 | 9066 | T | C | - | 6.90 |
| NS2B | 4335 | A | G | - | 3.11 | NS5 | 9079 | T | C | - | 5.49 |
| NS2B | 4338 | G | A | - | 5.13 | NS5 | 9322 | T | C | Ser585Pro | 22.73 |
| NS2B | 4341 | C | T | - | 7.04 | NS5 | 9477 | T | C | - | 22.47 |
| NS2B | 4350 | C | T | - | 5.73 | NS5 | 9501 | C | G | His644Gln | 7.25 |
| NS2B | 4482 | A | G | - | 15.07 | NS5 | 9576 | C | T | - | 12.68 |
| NS2B | 4491 | G | A | - | 6.25 | NS5 | 9942 | T | C | - | 5.79 |
| NS2B | 4509 | T | G | - | 8.40 | NS5 | 9969 | G | A | - | 7.41 |
| NS3 | 4557 | A | C | - | 5.17 | NS5 | 9984 | G | A | - | 7.32 |
| NS3 | 4566 | A | G | - | 6.78 | NS5 | 9997 | C | A | Leu810Met | 7.55 |
| NS3 | 4573 | C | T | - | 6.72 | NS5 | 10083 | T | C | - | 6.42 |
| NS3 | 4596 | T | C | - | 6.72 | NS5 | 10155 | C | T | - | 5.26 |
| NS3 | 4602 | A | G | - | 6.03 | NS5 | 10202 | A | G | Glu878Gly | 4.76 |
| NS3 | 4604 | G | A | Arg28Lys | 5.93 | NS5 | 10253 | T | A | Met895Lys | 18.92 |
| NS3 | 4620 | T | C | - | 7.69 | 3UTR | 10389 | CA | C | - | 8.39 |
| NS3 | 4623 | T | C | - | 5.83 | 3UTR | 10389 | C | CA | - | 4.52 |
| NS3 | 4635 | C | T | - | 6.90 | 3UTR | 10408 | C | T | - | 6.37 |
| NS3 | 4641 | T | C | - | 8.33 | 3UTR | 10444 | T | TA | - | 5.62 |

| **194** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SD | | | | | | NS3 | 4974 | T | C | - | 15.50 |
| Primary | | | | | | NS3 | 4983 | T | C | - | 15.57 |
| 3.16E+03 | | | | | | NS3 | 5019 | C | T | - | 15.13 |
| 4 | | | | | | NS3 | 5079 | A | G | - | 19.78 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5190 | C | T | - | 14.94 |
| C | 111 | A | G | - | 11.94 | NS3 | 5235 | T | C | - | 26.12 |
| C | 219 | C | T | - | 6.06 | NS3 | 5304 | T | C | - | 11.84 |
| C | 270 | A | G | - | 14.53 | NS3 | 5319 | T | C | - | 7.07 |
| C | 327 | T | C | - | 5.15 | NS3 | 5340 | C | T | - | 11.22 |
| C | 333 | T | C | - | 18.56 | NS3 | 5394 | A | G | - | 31.82 |
| C | 350 | G | A | Arg85Lys | 18.26 | NS3 | 5643 | T | C | - | 33.96 |
| C | 384 | T | C | - | 25.69 | NS3 | 5649 | G | A | - | 14.29 |
| C | 411 | T | C | - | 20.83 | NS3 | 5673 | A | G | - | 28.30 |
| prM/M | 445 | C | T | - | 39.60 | NS3 | 5730 | T | C | - | 42.72 |
| prM/M | 450 | C | T | - | 12.95 | NS3 | 5763 | T | C | - | 20.62 |
| prM/M | 508 | C | T | - | 43.12 | NS3 | 5775 | G | A | - | 12.22 |
| prM/M | 531 | T | C | - | 3.94 | NS3 | 5793 | C | T | - | 11.36 |
| prM/M | 540 | T | C | - | 26.09 | NS3 | 5823 | A | G | - | 13.70 |
| prM/M | 567 | A | G | - | 32.77 | NS3 | 5835 | A | G | - | 19.72 |
| prM/M | 606 | C | T | - | 16.67 | NS3 | 5844 | G | A | - | 14.08 |
| prM/M | 651 | A | G | - | 16.46 | NS3 | 5901 | A | G | - | 28.57 |
| prM/M | 723 | C | T | - | 13.64 | NS3 | 5915 | C | CA | Asn467fs | 17.65 |
| prM/M | 792 | A | G | - | 25.96 | NS3 | 5979 | A | C | - | 8.82 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| prM/M | 798 | C | T | - | 24.79 | NS3 | 6069 | G | T | - | 48.00 |
| prM/M | 820 | T | C | - | 27.94 | NS3 | 6099 | A | G | - | 29.09 |
| prM/M | 834 | C | T | - | 18.40 | NS3 | 6105 | A | G | - | 24.53 |
| prM/M | 837 | C | T | - | 20.27 | NS3 | 6108 | A | G | - | 12.28 |
| prM/M | 852 | A | G | - | 21.23 | NS3 | 6157 | T | C | - | 8.33 |
| prM/M | 855 | C | T | - | 12.67 | NS3 | 6186 | C | T | - | 4.71 |
| prM/M | 856 | T | C | - | 21.48 | NS3 | 6324 | T | C | - | 24.58 |
| prM/M | 873 | C | A | - | 20.98 | NS3 | 6330 | C | T | - | 28.57 |
| prM/M | 921 | C | T | - | 22.22 | NS4A | 6384 | C | T | - | 14.39 |
| E | 954 | A | C | - | 15.67 | NS4A | 6423 | C | T | - | 20.17 |
| E | 1005 | A | C | - | 34.59 | NS4A | 6466 | C | T | - | 4.52 |
| E | 1023 | T | C | - | 6.54 | NS4A | 6471 | T | C | - | 7.32 |
| E | 1040 | C | CA | Asn37fs | 5.79 | NS4A | 6491 | G | A | Arg39Lys | 44.20 |
| E | 1102 | C | T | - | 35.53 | NS4A | 6495 | G | A | - | 22.76 |
| E | 1287 | T | C | - | 15.46 | NS4A | 6496 | T | C | Tyr41His | 47.20 |
| E | 1293 | C | T | - | 29.67 | NS4A | 6500 | C | A | Thr42Asn | 44.36 |
| E | 1302 | A | G | - | 31.25 | NS4A | 6512 | G | A | Ser46Asn | 14.67 |
| E | 1353 | C | T | - | 6.25 | NS4A | 6547 | C | T | - | 42.11 |
| E | 1368 | C | T | - | 8.08 | NS4A | 6556 | C | T | - | 41.73 |
| E | 1413 | C | A | - | 16.95 | NS4A | 6562 | G | A | Ala63Thr | 43.45 |
| E | 1473 | T | C | - | 16.05 | NS4A | 6582 | C | T | - | 5.29 |
| E | 1479 | C | T | - | 36.99 | NS4A | 6585 | A | G | - | 9.26 |
| E | 1599 | A | G | - | 9.40 | NS4A | 6588 | C | T | - | 4.14 |
| E | 1626 | T | C | - | 11.11 | NS4A | 6591 | A | G | - | 4.97 |
| E | 1653 | T | C | - | 10.98 | NS4A | 6606 | T | C | - | 22.29 |
| E | 1701 | T | C | - | 41.82 | NS4A | 6652 | A | G | Ile93Val | 22.79 |
| E | 1734 | G | A | - | 13.68 | NS4B | 6844 | G | GA | Thr9fs | 4.00 |
| E | 1842 | T | C | - | 13.46 | NS4B | 6864 | C | T | - | 8.85 |
| E | 1863 | G | A | - | 36.59 | NS4B | 6871 | G | A | Gly16Arg | 8.96 |
| E | 1920 | G | T | - | 12.07 | NS4B | 6879 | T | C | - | 42.28 |
| E | 1986 | C | T | - | 28.57 | NS4B | 6924 | T | C | - | 22.48 |
| E | 1995 | G | A | - | 35.04 | NS4B | 6957 | T | C | - | 5.71 |
| E | 2007 | C | T | - | 5.17 | NS4B | 6966 | T | C | - | 24.42 |
| E | 2064 | C | T | - | 14.86 | NS4B | 6982 | C | T | Arg53* | 3.23 |
| E | 2082 | A | G | - | 10.77 | NS4B | 6984 | A | G | - | 18.75 |
| E | 2223 | T | C | - | 21.38 | NS4B | 7008 | G | A | - | 18.28 |
| E | 2244 | C | T | - | 28.48 | NS4B | 7018 | C | T | - | 5.20 |
| E | 2256 | C | T | - | 10.69 | NS4B | 7038 | G | A | - | 48.78 |
| E | 2276 | C | T | Ala447Val | 33.33 | NS4B | 7041 | C | T | - | 22.27 |
| E | 2280 | T | C | - | 3.49 | NS4B | 7059 | T | C | - | 19.61 |
| E | 2319 | C | A | - | 14.21 | NS4B | 7083 | G | A | - | 5.61 |
| E | 2397 | G | A | - | 25.00 | NS4B | 7134 | C | T | - | 6.11 |
| NS1 | 2448 | A | G | - | 24.56 | NS4B | 7137 | T | C | - | 26.89 |
| NS1 | 2550 | T | C | - | 31.41 | NS4B | 7186 | A | G | ile121Val | 7.32 |
| NS1 | 2652 | T | C | - | 31.64 | NS4B | 7203 | A | G | - | 12.90 |
| NS1 | 2685 | T | C | - | 5.59 | NS4B | 7287 | C | T | - | 19.44 |
| NS1 | 2878 | C | A | Leu153Met | 37.19 | NS4B | 7449 | T | C | - | 9.89 |
| NS1 | 2898 | T | C | - | 33.87 | NS4B | 7515 | G | A | - | 8.93 |
| NS1 | 2951 | T | C | Val177Ala | 41.13 | NS4B | 7542 | C | T | - | 17.95 |
| NS1 | 2961 | C | T | - | 37.27 | NS5 | 7655 | A | G | Lys29Arg | 22.58 |
| NS1 | 2976 | A | G | - | 42.72 | NS5 | 7656 | G | A | - | 30.30 |
| NS1 | 2985 | T | A | - | 12.17 | NS5 | 7719 | G | A | - | 8.89 |
| NS1 | 3009 | C | T | - | 7.50 | NS5 | 7976 | C | A | Pro136Gln | 10.60 |
| NS1 | 3114 | C | A | - | 22.67 | NS5 | 7986 | G | A | - | 8.48 |
| NS1 | 3162 | T | A | Phe247Leu | 12.82 | NS5 | 7999 | T | C | - | 46.58 |
| NS1 | 3241 | G | GCCT | Glu274AlaTer | 1.97 | NS5 | 8103 | T | C | - | 6.17 |
| NS1 | 3300 | T | C | - | 3.94 | NS5 | 8298 | C | T | - | 11.39 |
| NS1 | 3330 | C | T | - | 17.75 | NS5 | 8310 | T | C | - | 42.03 |
| NS1 | 3339 | G | A | - | 20.25 | NS5 | 8395 | G | A | Val276Ile | 50.00 |
| NS1 | 3342 | C | T | - | 4.49 | NS5 | 8395 | A | G | Ile276Val | 50.00 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2A | 3489 | G | A | - | 20.63 | NS5 | 8424 | A | G | - | 30.77 |
| NS2A | 3562 | A | G | Thr29Ala | 5.66 | NS5 | 8448 | T | C | - | 25.00 |
| NS2A | 3595 | T | C | Phe40Leu | 6.50 | NS5 | 8562 | T | C | - | 6.45 |
| NS2A | 3612 | T | A | - | 3.52 | NS5 | 8574 | C | T | - | 31.52 |
| NS2A | 3663 | T | C | - | 5.52 | NS5 | 8604 | C | T | - | 17.31 |
| NS2A | 3684 | T | C | - | 15.21 | NS5 | 8640 | G | A | - | 26.67 |
| NS2A | 3720 | C | CA | Val83fs | 1.13 | NS5 | 8658 | T | C | - | 33.33 |
| NS2A | 3720 | C | T | - | 16.92 | NS5 | 8722 | C | T | - | 28.57 |
| NS2A | 3750 | A | G | - | 5.63 | NS5 | 8745 | A | G | - | 12.00 |
| NS2A | 3801 | C | T | - | 6.93 | NS5 | 8746 | A | G | Met393Val | 16.00 |
| NS2A | 3813 | C | T | - | 10.07 | NS5 | 8751 | T | C | - | 11.54 |
| NS2A | 3844 | C | T | - | 10.40 | NS5 | 8799 | G | T | - | 7.02 |
| NS2A | 3855 | G | A | - | 2.22 | NS5 | 8803 | G | A | Val412Ile | 40.35 |
| NS2A | 3876 | C | T | - | 8.20 | NS5 | 8823 | A | G | - | 14.29 |
| NS2A | 3910 | T | C | - | 5.28 | NS5 | 9006 | T | C | - | 6.15 |
| NS2A | 4034 | A | G | Gln186Arg | 18.70 | NS5 | 9015 | C | T | - | 20.29 |
| NS2A | 4063 | T | C | - | 23.23 | NS5 | 9042 | T | C | - | 9.33 |
| NS2A | 4110 | T | C | - | 15.56 | NS5 | 9303 | G | A | - | 33.33 |
| NS2B | 4206 | T | C | - | 23.08 | NS5 | 9309 | G | A | - | 50.00 |
| NS2B | 4249 | G | A | Val40Ile | 5.50 | NS5 | 9318 | A | G | - | 29.41 |
| NS2B | 4251 | A | G | - | 8.08 | NS5 | 9345 | C | T | - | 25.00 |
| NS2B | 4281 | T | C | - | 9.30 | NS5 | 9375 | G | A | - | 13.04 |
| NS2B | 4335 | A | G | - | 14.12 | NS5 | 9384 | C | T | - | 19.23 |
| NS2B | 4350 | C | T | - | 19.18 | NS5 | 9399 | T | C | - | 27.59 |
| NS2B | 4479 | T | C | - | 22.09 | NS5 | 9576 | T | C | - | 9.49 |
| NS2B | 4482 | G | A | - | 13.79 | NS5 | 9628 | G | A | Val687Ile | 5.79 |
| NS3 | 4539 | C | T | - | 16.67 | NS5 | 9660 | A | T | - | 12.09 |
| NS3 | 4557 | A | C | - | 10.67 | NS5 | 9725 | A | T | Lys719Ile | 27.82 |
| NS3 | 4562 | G | A | Gly14Glu | 13.70 | NS5 | 9768 | A | G | - | 24.65 |
| NS3 | 4573 | C | T | - | 10.53 | NS5 | 9789 | T | C | - | 23.65 |
| NS3 | 4587 | C | T | - | 9.78 | NS5 | 9795 | G | A | - | 18.37 |
| NS3 | 4602 | A | G | - | 15.28 | NS5 | 9810 | T | C | - | 7.03 |
| NS3 | 4604 | G | A | Arg28Lys | 15.49 | NS5 | 9888 | A | G | - | 8.59 |
| NS3 | 4620 | T | C | - | 14.29 | NS5 | 9942 | T | C | - | 30.68 |
| NS3 | 4623 | T | C | - | 16.13 | NS5 | 10075 | G | A | Val836Ile | 21.90 |
| NS3 | 4629 | T | C | - | 14.29 | 3UTR | 10280 | A | G | - | 28.57 |
| NS3 | 4635 | C | T | - | 16.39 | 3UTR | 10389 | C | CA | - | 3.67 |
| NS3 | 4641 | T | C | - | 14.75 | 3UTR | 10389 | C | CAA | - | 30.28 |
| NS3 | 4707 | G | A | - | 8.62 | 3UTR | 10389 | C | T | - | 18.18 |
| NS3 | 4764 | A | G | - | 20.59 | 3UTR | 10407 | C | T | - | 49.22 |
| NS3 | 4806 | C | T | - | 8.86 | 3UTR | 10443 | T | TA | - | 2.11 |

| **195** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SD | | | | | | NS3 | 5026 | GA | G | Ser171fs | 3.57 |
| Primary | | | | | | NS3 | 5046 | T | C | - | 17.86 |
| 7.17E+01 | | | | | | NS3 | 5055 | C | T | - | 14.29 |
| 4 | | | | | | NS3 | 5067 | T | C | - | 17.24 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5073 | A | G | - | 13.79 |
| C | 168 | A | G | - | 13.73 | NS3 | 5076 | G | A | - | 12.50 |
| C | 181 | C | T | - | 13.33 | NS3 | 5078 | A | G | Lys186Arg | 12.31 |
| C | 201 | T | C | - | 45.10 | NS3 | 5091 | C | T | - | 12.12 |
| C | 213 | A | G | - | 11.32 | NS3 | 5103 | T | C | - | 14.08 |
| C | 219 | C | A | - | 11.32 | NS3 | 5112 | A | G | - | 20.69 |
| C | 228 | A | G | - | 10.71 | NS3 | 5121 | A | G | - | 5.17 |
| C | 246 | T | G | - | 11.76 | NS3 | 5139 | C | A | - | 7.35 |
| C | 252 | G | A | - | 11.54 | NS3 | 5190 | C | T | - | 25.32 |
| C | 294 | A | G | - | 11.76 | NS3 | 5235 | C | T | - | 48.48 |
| C | 318 | A | G | - | 11.32 | NS3 | 5256 | A | C | - | 20.45 |
| C | 324 | A | G | - | 11.76 | NS3 | 5268 | C | T | - | 28.57 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 327 | T | C | - | 36.00 | NS3 | 5277 | C | T | - | 46.15 |
| C | 336 | T | C | - | 11.11 | NS3 | 5283 | A | G | - | 40.63 |
| C | 345 | G | C | - | 8.47 | NS3 | 5286 | G | A | - | 15.63 |
| C | 375 | C | T | - | 12.12 | NS3 | 5295 | C | T | - | 20.51 |
| C | 384 | C | T | - | 41.67 | NS3 | 5296 | C | T | - | 20.51 |
| C | 387 | G | A | - | 32.14 | NS3 | 5304 | T | C | - | 41.67 |
| C | 411 | T | C | - | 5.80 | NS3 | 5319 | T | C | - | 29.79 |
| prM/M | 445 | T | C | - | 38.20 | NS3 | 5331 | A | G | - | 21.82 |
| prM/M | 450 | C | T | - | 21.43 | NS3 | 5334 | A | G | - | 21.82 |
| prM/M | 480 | T | C | - | 10.98 | NS3 | 5532 | A | G | - | 15.15 |
| prM/M | 481 | G | A | Gly15Ser | 11.11 | NS3 | 5544 | G | A | - | 13.89 |
| prM/M | 483 | T | C | - | 11.69 | NS3 | 5550 | A | G | - | 15.38 |
| prM/M | 486 | G | A | - | 11.54 | NS3 | 5559 | A | T | - | 13.04 |
| prM/M | 489 | G | A | - | 11.84 | NS3 | 5574 | C | T | - | 11.32 |
| prM/M | 507 | T | C | - | 12.66 | NS3 | 5610 | A | G | - | 17.65 |
| prM/M | 513 | C | T | - | 12.82 | NS3 | 5667 | G | A | - | 13.04 |
| prM/M | 531 | T | G | - | 9.88 | NS3 | 5730 | C | T | - | 26.67 |
| prM/M | 567 | G | A | - | 45.16 | NS3 | 5781 | G | A | - | 10.26 |
| prM/M | 606 | C | T | - | 36.54 | NS3 | 5796 | T | C | - | 37.21 |
| prM/M | 609 | A | G | - | 9.80 | NS3 | 5799 | A | G | - | 13.33 |
| prM/M | 612 | A | G | - | 10.20 | NS3 | 5802 | C | T | - | 47.62 |
| prM/M | 633 | T | C | - | 15.09 | NS3 | 5817 | T | C | - | 21.74 |
| prM/M | 651 | G | A | - | 36.84 | NS3 | 5829 | T | C | - | 30.00 |
| prM/M | 795 | T | C | - | 9.43 | NS3 | 5832 | C | T | - | 30.77 |
| prM/M | 797 | T | C | Val120Ala | 9.43 | NS3 | 5835 | A | G | - | 15.79 |
| prM/M | 837 | C | T | - | 41.67 | NS3 | 5841 | G | A | - | 19.44 |
| prM/M | 864 | C | T | - | 44.78 | NS3 | 5844 | G | A | - | 17.14 |
| prM/M | 873 | A | C | - | 42.37 | NS3 | 5847 | C | T | - | 37.14 |
| prM/M | 876 | G | A | - | 25.00 | NS3 | 5859 | T | C | - | 24.24 |
| prM/M | 880 | C | T | His148Tyr | 23.81 | NS3 | 5868 | G | A | - | 18.18 |
| prM/M | 882 | C | T | - | 22.06 | NS3 | 5886 | G | A | - | 20.00 |
| prM/M | 891 | G | A | - | 29.85 | NS3 | 5915 | C | CA | Asn467fs | 12.50 |
| prM/M | 893 | C | T | Ala152Val | 32.31 | NS3 | 6009 | T | C | - | 46.67 |
| prM/M | 894 | C | A | - | 34.85 | NS3 | 6048 | C | T | - | 12.96 |
| prM/M | 921 | C | T | - | 27.54 | NS3 | 6063 | A | G | - | 20.93 |
| E | 954 | A | C | - | 24.73 | NS3 | 6066 | A | G | - | 20.93 |
| E | 960 | T | C | - | 21.51 | NS3 | 6069 | G | T | - | 39.02 |
| E | 969 | C | T | - | 18.68 | NS3 | 6087 | A | G | - | 28.26 |
| E | 972 | A | G | - | 17.39 | NS3 | 6093 | T | C | - | 25.64 |
| E | 1008 | T | C | - | 16.42 | NS3 | 6094 | T | C | - | 43.59 |
| E | 1023 | T | C | - | 18.06 | NS3 | 6096 | G | A | - | 25.64 |
| E | 1032 | G | A | - | 11.27 | NS3 | 6099 | A | G | - | 32.50 |
| E | 1062 | T | C | - | 9.72 | NS3 | 6108 | A | G | - | 23.40 |
| E | 1068 | A | G | - | 9.68 | NS3 | 6141 | G | A | - | 21.43 |
| E | 1102 | C | T | - | 46.34 | NS3 | 6157 | T | C | - | 39.62 |
| E | 1122 | A | G | - | 19.35 | NS3 | 6167 | A | G | Lys549Arg | 22.22 |
| E | 1148 | A | C | Glu71Ala | 23.08 | NS3 | 6177 | T | C | - | 17.86 |
| E | 1152 | G | T | - | 25.00 | NS3 | 6186 | C | T | - | 8.62 |
| E | 1155 | T | A | - | 25.00 | NS3 | 6198 | C | T | - | 10.42 |
| E | 1287 | T | C | - | 11.54 | NS3 | 6220 | A | G | Ile567Val | 30.56 |
| E | 1353 | C | T | - | 15.79 | NS3 | 6222 | T | C | - | 30.56 |
| E | 1413 | C | A | - | 14.81 | NS3 | 6238 | C | T | - | 7.50 |
| E | 1415 | A | G | Lys160Arg | 26.92 | NS3 | 6279 | G | A | - | 8.89 |
| E | 1509 | C | T | - | 8.51 | NS3 | 6288 | A | G | - | 12.50 |
| E | 1686 | C | T | - | 12.50 | NS3 | 6294 | A | G | - | 10.00 |
| E | 1692 | C | T | - | 12.77 | NS3 | 6300 | C | T | - | 23.81 |
| E | 1698 | G | A | - | 12.24 | NS3 | 6303 | A | G | - | 11.36 |
| E | 1710 | G | T | - | 14.29 | NS3 | 6307 | T | C | - | 10.20 |
| E | 1713 | C | T | - | 13.73 | NS3 | 6312 | T | C | - | 9.80 |
| E | 1719 | C | T | - | 10.00 | NS3 | 6330 | C | T | - | 48.68 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1722 | G | A | - | 12.77 | NS3 | 6333 | A | G | - | 7.79 |
| E | 1858 | A | G | Ile308Val | 41.38 | NS3 | 6339 | A | G | - | 7.32 |
| E | 1860 | T | C | - | 50.00 | NS3 | 6342 | A | C | - | 7.14 |
| E | 1923 | C | T | - | 26.47 | NS3 | 6354 | G | A | - | 9.09 |
| E | 1938 | G | A | - | 18.18 | NS3 | 6357 | A | G | - | 9.09 |
| E | 1955 | C | T | Thr340Ile | 16.67 | NS3 | 6366 | T | C | - | 12.94 |
| E | 1956 | A | G | - | 17.07 | NS4A | 6378 | T | C | - | 40.51 |
| E | 1959 | T | C | - | 19.05 | NS4A | 6379 | T | C | - | 40.51 |
| E | 1960 | T | C | - | 16.67 | NS4A | 6390 | C | T | - | 13.95 |
| E | 1974 | C | T | - | 13.21 | NS4A | 6423 | C | T | - | 14.55 |
| E | 1983 | A | T | - | 6.90 | NS4A | 6429 | T | C | - | 15.69 |
| E | 2007 | C | T | - | 10.42 | NS4A | 6435 | G | A | - | 13.46 |
| E | 2055 | C | T | - | 25.00 | NS4A | 6444 | C | T | - | 10.42 |
| E | 2082 | A | G | - | 22.22 | NS4A | 6448 | C | T | - | 9.26 |
| E | 2223 | T | C | - | 45.68 | NS4A | 6462 | T | C | - | 30.77 |
| E | 2241 | T | C | - | 11.11 | NS4A | 6471 | T | C | - | 48.21 |
| E | 2250 | A | G | - | 9.46 | NS4A | 6491 | A | G | Lys39Arg | 42.37 |
| E | 2256 | C | T | - | 37.18 | NS4A | 6496 | T | C | Tyr41His | 37.29 |
| E | 2276 | C | T | Ala447Val | 13.25 | NS4A | 6500 | C | A | Thr42Asn | 33.33 |
| E | 2280 | T | C | - | 6.82 | NS4A | 6519 | G | C | - | 8.06 |
| E | 2283 | T | C | - | 6.59 | NS4A | 6528 | T | C | - | 26.15 |
| E | 2286 | G | A | - | 8.24 | NS4A | 6547 | C | T | - | 22.22 |
| E | 2289 | C | T | - | 39.56 | NS4A | 6556 | C | T | - | 33.33 |
| E | 2298 | T | C | - | 9.64 | NS4A | 6562 | G | A | Ala63Thr | 33.82 |
| E | 2307 | C | T | - | 40.00 | NS4A | 6564 | A | G | - | 49.21 |
| E | 2319 | C | A | - | 26.67 | NS4A | 6585 | G | A | - | 31.87 |
| E | 2319 | C | T | - | 8.89 | NS4A | 6599 | GA | G | Arg76fs | 2.06 |
| E | 2320 | A | G | Ile462Val | 8.51 | NS4A | 6615 | G | A | - | 6.25 |
| E | 2328 | A | T | - | 28.87 | NS4A | 6633 | T | C | - | 31.25 |
| E | 2349 | T | C | - | 7.77 | NS4A | 6645 | A | G | - | 6.41 |
| E | 2367 | G | A | - | 13.10 | NS4A | 6651 | C | T | - | 6.76 |
| E | 2371 | C | T | - | 12.35 | NS4A | 6654 | T | C | - | 27.50 |
| E | 2379 | G | A | - | 13.85 | NS4A | 6666 | T | C | - | 18.06 |
| E | 2395 | C | T | - | 16.98 | NS4A | 6678 | A | G | - | 16.67 |
| E | 2400 | C | T | - | 12.50 | NS4A | 6681 | A | G | - | 23.53 |
| E | 2401 | C | T | - | 14.55 | NS4A | 6693 | A | G | - | 21.15 |
| E | 2409 | T | C | - | 12.50 | NS4A | 6711 | G | GT | Leu115fs | 12.96 |
| NS1 | 2433 | C | T | - | 18.75 | NS4A | 6732 | C | T | - | 9.09 |
| NS1 | 2442 | C | T | - | 18.46 | NS4A | 6777 | G | A | - | 27.08 |
| NS1 | 2448 | A | G | - | 42.86 | NS4A | 6780 | C | T | - | 26.53 |
| NS1 | 2481 | C | T | - | 8.57 | NS4B | 6837 | T | G | - | 19.18 |
| NS1 | 2517 | T | C | - | 7.50 | NS4B | 6840 | C | T | - | 19.72 |
| NS1 | 2520 | G | A | - | 7.41 | NS4B | 6841 | C | T | - | 34.25 |
| NS1 | 2540 | CA | C | Lys41fs | 2.50 | NS4B | 6844 | G | GA | Thr9fs | 2.60 |
| NS1 | 2589 | A | G | - | 42.25 | NS4B | 6852 | C | G | - | 21.79 |
| NS1 | 2667 | T | C | - | 8.57 | NS4B | 6861 | C | T | - | 17.95 |
| NS1 | 2724 | A | G | - | 15.69 | NS4B | 6862 | C | T | Leu13Phe | 18.42 |
| NS1 | 2731 | T | C | - | 20.45 | NS4B | 6864 | C | T | - | 30.77 |
| NS1 | 2760 | C | T | - | 18.97 | NS4B | 6870 | T | G | Phe15Leu | 18.82 |
| NS1 | 2778 | A | C | - | 18.46 | NS4B | 6871 | G | A | Gly16Arg | 39.53 |
| NS1 | 2802 | GTCT | G | Ser128del | 6.25 | NS4B | 6879 | T | C | - | 23.66 |
| NS1 | 2808 | C | T | - | 13.33 | NS4B | 6880 | A | G | Thr19Ala | 19.79 |
| NS1 | 2835 | T | C | - | 14.52 | NS4B | 6889 | G | C | Glu22Gln | 14.89 |
| NS1 | 2878 | C | A | Leu153Met | 40.38 | NS4B | 6891 | A | G | - | 15.96 |
| NS1 | 2880 | G | A | - | 13.21 | NS4B | 6892 | T | C | Ser23Pro | 14.58 |
| NS1 | 2898 | T | C | - | 49.12 | NS4B | 6894 | T | C | - | 14.58 |
| NS1 | 2952 | G | A | - | 41.94 | NS4B | 6924 | T | C | - | 8.74 |
| NS1 | 2961 | C | T | - | 40.68 | NS4B | 6942 | G | C | - | 13.59 |
| NS1 | 2966 | A | G | Lys182Arg | 16.39 | NS4B | 6948 | T | C | - | 30.39 |
| NS1 | 2976 | A | G | - | 39.13 | NS4B | 6957 | T | C | - | 18.18 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS1 | 2989 | G | A | Asp190Asn | 5.45 | NS4B | 6967 | G | A | Val48Ile | 14.41 |
| NS1 | 2991 | T | C | - | 41.51 | NS4B | 6979 | T | C | - | 10.29 |
| NS1 | 3001 | G | A | Val194Ile | 5.36 | NS4B | 6990 | C | T | - | 13.79 |
| NS1 | 3033 | T | C | - | 7.58 | NS4B | 7008 | G | A | - | 39.24 |
| NS1 | 3057 | G | A | Met212Ile | 13.64 | NS4B | 7014 | C | G | - | 17.65 |
| NS1 | 3060 | G | A | - | 12.12 | NS4B | 7017 | C | T | - | 17.88 |
| NS1 | 3102 | G | A | - | 14.04 | NS4B | 7029 | T | A | - | 38.06 |
| NS1 | 3111 | C | T | - | 12.96 | NS4B | 7032 | T | C | - | 18.80 |
| NS1 | 3114 | C | A | - | 40.68 | NS4B | 7038 | A | G | - | 7.69 |
| NS1 | 3120 | C | T | - | 15.09 | NS4B | 7059 | T | C | - | 33.88 |
| NS1 | 3129 | A | G | - | 14.81 | NS4B | 7062 | G | A | - | 40.65 |
| NS1 | 3130 | T | C | - | 12.96 | NS4B | 7083 | G | A | - | 19.01 |
| NS1 | 3152 | C | CA | Asn246fs | 6.52 | NS4B | 7089 | C | T | - | 11.86 |
| NS1 | 3156 | A | G | - | 15.56 | NS4B | 7092 | C | T | - | 14.29 |
| NS1 | 3165 | C | T | - | 15.91 | NS4B | 7101 | C | T | - | 7.92 |
| NS1 | 3168 | G | A | - | 15.22 | NS4B | 7104 | C | T | - | 8.00 |
| NS1 | 3174 | G | A | - | 9.80 | NS4B | 7107 | T | C | - | 9.47 |
| NS1 | 3186 | C | T | - | 13.21 | NS4B | 7110 | C | T | - | 8.16 |
| NS1 | 3201 | C | T | - | 10.17 | NS4B | 7116 | A | G | - | 12.24 |
| NS1 | 3204 | T | C | - | 10.17 | NS4B | 7122 | C | T | - | 11.70 |
| NS1 | 3213 | A | G | - | 10.17 | NS4B | 7143 | C | T | - | 18.33 |
| NS1 | 3222 | T | C | - | 9.68 | NS4B | 7146 | C | T | - | 17.54 |
| NS1 | 3234 | T | C | - | 44.44 | NS4B | 7155 | T | C | - | 18.18 |
| NS1 | 3258 | C | T | - | 10.00 | NS4B | 7161 | T | C | - | 16.33 |
| NS1 | 3261 | C | T | - | 10.00 | NS4B | 7165 | T | C | - | 38.00 |
| NS1 | 3270 | T | C | - | 8.91 | NS4B | 7185 | T | C | - | 17.14 |
| NS1 | 3324 | C | T | - | 5.26 | NS4B | 7200 | T | C | - | 24.24 |
| NS1 | 3330 | C | T | - | 27.08 | NS4B | 7206 | A | G | - | 22.86 |
| NS1 | 3339 | G | A | - | 8.33 | NS4B | 7248 | C | T | - | 38.46 |
| NS1 | 3357 | C | T | - | 9.09 | NS4B | 7263 | A | T | - | 19.23 |
| NS1 | 3366 | C | T | - | 10.77 | NS4B | 7266 | C | G | - | 19.23 |
| NS1 | 3402 | T | C | - | 34.25 | NS4B | 7411 | C | T | - | 9.09 |
| NS2A | 3489 | G | A | - | 20.51 | NS4B | 7435 | C | T | - | 7.69 |
| NS2A | 3526 | C | T | - | 15.15 | NS5 | 7582 | G | A | Val5Ile | 40.91 |
| NS2A | 3546 | T | C | - | 18.42 | NS5 | 7608 | GA | G | Ser15fs | 8.70 |
| NS2A | 3624 | T | C | - | 47.46 | NS5 | 7656 | G | A | - | 17.24 |
| NS2A | 3629 | G | A | Arg51Lys | 48.33 | NS5 | 7725 | C | T | - | 21.43 |
| NS2A | 3734 | CT | C | Phe87fs | 1.55 | NS5 | 7728 | T | C | - | 23.08 |
| NS2A | 3743 | C | T | Ala89Val | 26.72 | NS5 | 7737 | A | G | - | 40.00 |
| NS2A | 3753 | C | T | - | 38.36 | NS5 | 7743 | A | C | - | 40.00 |
| NS2A | 3768 | C | T | - | 36.73 | NS5 | 7752 | A | G | - | 28.57 |
| NS2A | 3801 | C | T | - | 12.58 | NS5 | 7776 | C | T | - | 29.41 |
| NS2A | 3825 | G | A | - | 4.00 | NS5 | 7777 | A | C | Met70Leu | 37.50 |
| NS2A | 3834 | C | T | - | 8.78 | NS5 | 7788 | G | A | - | 40.00 |
| NS2A | 3837 | T | A | - | 7.43 | NS5 | 7800 | G | A | - | 25.00 |
| NS2A | 3840 | A | T | - | 7.47 | NS5 | 7806 | T | C | - | 30.77 |
| NS2A | 3858 | A | G | - | 11.45 | NS5 | 7809 | T | C | - | 28.57 |
| NS2A | 3874 | G | A | Val133Ile | 9.87 | NS5 | 7848 | A | C | - | 25.93 |
| NS2A | 3876 | C | T | - | 49.00 | NS5 | 7890 | A | G | - | 19.64 |
| NS2A | 3879 | C | A | - | 43.24 | NS5 | 7899 | C | T | - | 18.18 |
| NS2A | 3900 | A | G | - | 11.59 | NS5 | 7908 | C | T | - | 14.71 |
| NS2A | 3903 | A | G | - | 11.73 | NS5 | 7914 | C | A | - | 13.24 |
| NS2A | 3906 | C | T | - | 27.67 | NS5 | 7947 | G | T | - | 10.87 |
| NS2A | 3910 | T | C | - | 16.34 | NS5 | 7956 | G | A | - | 13.48 |
| NS2A | 3912 | G | A | - | 12.20 | NS5 | 7971 | C | T | - | 16.47 |
| NS2A | 3921 | T | C | - | 33.92 | NS5 | 7999 | T | C | - | 29.67 |
| NS2A | 3935 | C | T | Ser153Leu | 14.97 | NS5 | 8035 | A | G | Ile156Val | 12.94 |
| NS2A | 3936 | G | A | - | 14.97 | NS5 | 8037 | A | T | - | 11.76 |
| NS2A | 3942 | C | T | - | 16.38 | NS5 | 8064 | C | A | - | 14.29 |
| NS2A | 3958 | T | C | - | 12.78 | NS5 | 8070 | A | G | - | 18.46 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2A | 3960 | G | A | - | 13.37 | NS5 | 8083 | C | T | - | 40.68 |
| NS2A | 3961 | CA | C | Asn163fs | 1.21 | NS5 | 8088 | C | T | - | 15.15 |
| NS2A | 3966 | C | T | - | 15.20 | NS5 | 8091 | T | C | - | 14.71 |
| NS2A | 3975 | G | A | - | 13.17 | NS5 | 8103 | T | C | - | 33.33 |
| NS2A | 3981 | T | C | - | 15.09 | NS5 | 8115 | C | T | - | 16.95 |
| NS2A | 3991 | T | C | - | 17.47 | NS5 | 8127 | T | C | - | 29.31 |
| NS2A | 3998 | C | T | Ala174Val | 17.65 | NS5 | 8172 | T | C | - | 9.80 |
| NS2A | 4009 | T | A | Ser178Thr | 18.99 | NS5 | 8181 | C | T | - | 7.69 |
| NS2A | 4021 | C | T | - | 17.07 | NS5 | 8203 | C | A | - | 14.81 |
| NS2A | 4034 | A | G | Gln186Arg | 46.00 | NS5 | 8208 | C | T | - | 10.53 |
| NS2A | 4047 | T | C | - | 17.65 | NS5 | 8214 | G | A | - | 13.46 |
| NS2A | 4057 | C | T | - | 14.58 | NS5 | 8220 | A | G | - | 12.96 |
| NS2A | 4063 | T | C | - | 28.00 | NS5 | 8226 | C | T | - | 15.52 |
| NS2A | 4071 | A | C | - | 14.52 | NS5 | 8235 | T | C | - | 23.53 |
| NS2A | 4074 | A | G | - | 15.25 | NS5 | 8241 | T | C | - | 40.00 |
| NS2A | 4098 | T | C | - | 14.91 | NS5 | 8242 | A | T | Thr225Ser | 22.92 |
| NS2A | 4107 | T | C | - | 17.12 | NS5 | 8284 | T | C | - | 31.37 |
| NS2A | 4110 | T | C | - | 24.53 | NS5 | 8289 | T | C | - | 21.57 |
| NS2A | 4113 | G | A | - | 15.32 | NS5 | 8298 | C | T | - | 22.73 |
| NS2A | 4121 | G | A | Ser215Asn | 16.98 | NS5 | 8306 | A | G | Lys246Arg | 20.93 |
| NS2B | 4134 | C | T | - | 16.24 | NS5 | 8310 | T | C | - | 18.60 |
| NS2B | 4140 | G | A | - | 17.76 | NS5 | 8316 | A | G | - | 19.44 |
| NS2B | 4149 | A | G | - | 13.16 | NS5 | 8481 | T | C | - | 12.12 |
| NS2B | 4164 | C | T | - | 46.36 | NS5 | 8496 | T | C | - | 11.63 |
| NS2B | 4197 | A | G | - | 9.71 | NS5 | 8508 | A | G | - | 18.60 |
| NS2B | 4206 | T | C | - | 44.12 | NS5 | 8526 | T | C | - | 12.28 |
| NS2B | 4212 | T | C | - | 9.52 | NS5 | 8535 | C | T | - | 14.55 |
| NS2B | 4225 | T | C | - | 7.48 | NS5 | 8541 | G | A | - | 12.73 |
| NS2B | 4242 | C | T | - | 9.43 | NS5 | 8572 | G | A | Val335Ile | 22.64 |
| NS2B | 4248 | C | T | - | 9.80 | NS5 | 8574 | C | T | - | 21.82 |
| NS2B | 4249 | G | A | Val40Ile | 7.77 | NS5 | 8577 | T | C | - | 22.00 |
| NS2B | 4251 | A | G | - | 9.52 | NS5 | 8604 | C | T | - | 30.43 |
| NS2B | 4257 | C | T | - | 8.08 | NS5 | 8610 | T | C | - | 23.81 |
| NS2B | 4270 | C | A | - | 12.35 | NS5 | 8634 | C | T | - | 27.27 |
| NS2B | 4305 | T | C | - | 15.52 | NS5 | 8658 | C | T | - | 30.00 |
| NS2B | 4310 | A | G | Lys60Arg | 12.07 | NS5 | 8799 | G | A | - | 9.68 |
| NS2B | 4323 | G | A | - | 13.46 | NS5 | 8803 | G | A | Val412Ile | 36.67 |
| NS2B | 4335 | A | G | - | 18.00 | NS5 | 8823 | A | G | - | 15.79 |
| NS2B | 4341 | C | T | - | 35.29 | NS5 | 8832 | G | T | - | 12.20 |
| NS2B | 4350 | C | T | - | 47.50 | NS5 | 8967 | C | T | - | 42.86 |
| NS2B | 4368 | A | G | - | 22.22 | NS5 | 9042 | T | C | - | 42.86 |
| NS2B | 4386 | G | A | - | 31.03 | NS5 | 9063 | C | T | - | 11.76 |
| NS2B | 4401 | G | A | - | 20.69 | NS5 | 9071 | G | A | Gly501Glu | 10.00 |
| NS2B | 4434 | G | A | - | 13.33 | NS5 | 9079 | C | T | - | 44.44 |
| NS2B | 4482 | G | A | - | 27.66 | NS5 | 9112 | C | T | - | 26.67 |
| NS2B | 4491 | A | G | - | 38.30 | NS5 | 9508 | T | C | - | 7.69 |
| NS2B | 4509 | G | T | - | 33.33 | NS5 | 9532 | C | T | - | 10.71 |
| NS2B | 4512 | G | A | - | 13.21 | NS5 | 9561 | C | T | - | 6.67 |
| NS3 | 4539 | C | T | - | 20.00 | NS5 | 9576 | T | C | - | 30.51 |
| NS3 | 4542 | C | T | - | 20.93 | NS5 | 9628 | A | G | Ile687Val | 45.83 |
| NS3 | 4557 | A | C | - | 23.40 | NS5 | 9708 | T | C | - | 9.21 |
| NS3 | 4566 | A | G | - | 30.43 | NS5 | 9718 | G | A | Val717Ile | 10.39 |
| NS3 | 4573 | C | T | - | 29.55 | NS5 | 9726 | AG | A | Asp720fs | 2.70 |
| NS3 | 4596 | C | T | - | 37.78 | NS5 | 9795 | G | A | - | 41.27 |
| NS3 | 4602 | A | G | - | 26.09 | NS5 | 9840 | T | C | - | 40.54 |
| NS3 | 4604 | G | A | Arg28Lys | 25.00 | NS5 | 9849 | A | G | - | 39.74 |
| NS3 | 4620 | T | C | - | 21.15 | NS5 | 9857 | C | G | Thr763Ser | 6.90 |
| NS3 | 4623 | T | C | - | 18.52 | NS5 | 9870 | C | T | - | 10.71 |
| NS3 | 4629 | T | C | - | 16.98 | NS5 | 9889 | T | C | - | 18.06 |
| NS3 | 4635 | C | T | - | 20.00 | NS5 | 9900 | T | C | - | 22.81 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS3 | 4641 | T | C | - | 19.57 | NS5 | 9903 | C | T | - | 23.21 |
| NS3 | 4662 | T | C | - | 23.40 | NS5 | 9921 | G | A | - | 24.00 |
| NS3 | 4695 | G | A | - | 30.77 | NS5 | 9927 | T | C | - | 22.73 |
| NS3 | 4703 | G | A | Arg61Lys | 29.41 | NS5 | 10071 | A | T | Glu834Asp | 22.73 |
| NS3 | 4739 | A | G | Lys73Arg | 24.00 | NS5 | 10083 | C | T | - | 40.35 |
| NS3 | 4755 | A | G | - | 14.81 | NS5 | 10089 | G | A | - | 10.17 |
| NS3 | 4800 | G | A | - | 15.22 | 3UTR | 10369 | A | T | - | 13.64 |
| NS3 | 4806 | C | T | - | 18.75 | 3UTR | 10387 | C | T | - | 10.87 |
| NS3 | 4809 | A | G | - | 15.38 | 3UTR | 10389 | C | CA | - | 22.50 |
| NS3 | 4813 | C | T | - | 12.96 | 3UTR | 10389 | C | CAA | - | 10.00 |
| NS3 | 4824 | A | G | - | 10.53 | 3UTR | 10389 | C | T | - | 12.50 |
| NS3 | 4829 | G | GA | Asn105fs | 3.39 | 3UTR | 10400 | G | A | - | 11.90 |
| NS3 | 4830 | A | G | - | 45.76 | 3UTR | 10407 | C | T | - | 36.17 |
| NS3 | 4833 | A | G | - | 12.28 | 3UTR | 10411 | C | T | - | 16.07 |
| NS3 | 4845 | T | C | - | 9.23 | 3UTR | 10413 | G | A | - | 16.98 |
| NS3 | 4854 | A | G | - | 36.92 | 3UTR | 10450 | C | T | - | 12.24 |
| NS3 | 4864 | A | C | Ile115Leu | 6.45 | 3UTR | 10452 | C | T | - | 12.77 |
| NS3 | 4875 | C | T | - | 44.44 | 3UTR | 10463 | A | G | - | 11.32 |
| NS3 | 4974 | C | T | - | 44.90 | 3UTR | 10523 | A | G | - | 15.56 |
| NS3 | 5025 | C | T | - | 14.04 | 3UTR | 10540 | C | T | - | 20.00 |

| **196** | | | | | | NS3 | 4562 | G | A | Gly14Glu | 6.56 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SD | | | | | | NS3 | 4566 | A | G | - | 9.84 |
| Primary | | | | | | NS3 | 4573 | C | T | - | 10.00 |
| 3.89E+01 | | | | | | NS3 | 4602 | A | G | - | 9.26 |
| 5 | | | | | | NS3 | 4635 | C | T | - | 7.27 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 4812 | C | T | - | 44.44 |
| C | 111 | A | G | - | 18.75 | NS3 | 4830 | A | G | - | 40.32 |
| C | 127 | A | T | Thr11Ser | 13.73 | NS3 | 4905 | G | A | - | 32.32 |
| C | 201 | T | C | - | 34.19 | NS3 | 4944 | C | T | - | 31.46 |
| C | 231 | A | G | - | 7.02 | NS3 | 4959 | T | C | - | 41.11 |
| C | 315 | G | A | - | 23.91 | NS3 | 4971 | T | C | - | 47.96 |
| C | 327 | T | C | - | 10.20 | NS3 | 4974 | T | C | - | 11.70 |
| C | 384 | C | T | - | 33.33 | NS3 | 5070 | C | T | - | 38.75 |
| C | 387 | G | A | - | 33.33 | NS3 | 5082 | A | G | - | 45.24 |
| C | 411 | T | C | - | 4.04 | NS3 | 5190 | C | T | - | 18.69 |
| C | 431 | C | T | Ala112Val | 32.65 | NS3 | 5235 | T | C | - | 17.05 |
| prM/M | 445 | T | C | - | 34.51 | NS3 | 5277 | C | T | - | 35.29 |
| prM/M | 450 | C | T | - | 19.66 | NS3 | 5283 | A | G | - | 27.27 |
| prM/M | 508 | C | T | - | 46.73 | NS3 | 5304 | T | C | - | 20.27 |
| prM/M | 523 | G | A | Asp29Asn | 34.65 | NS3 | 5613 | T | C | - | 39.58 |
| prM/M | 525 | T | C | - | 35.00 | NS3 | 5649 | G | A | - | 21.95 |
| prM/M | 531 | T | C | - | 35.65 | NS3 | 5730 | C | T | - | 22.97 |
| prM/M | 567 | G | A | - | 41.38 | NS3 | 5775 | G | A | - | 11.76 |
| prM/M | 606 | C | T | - | 10.71 | NS3 | 5793 | C | T | - | 13.70 |
| prM/M | 651 | G | A | - | 28.21 | NS3 | 5823 | A | G | - | 17.19 |
| prM/M | 717 | A | G | - | 33.33 | NS3 | 5835 | A | G | - | 23.21 |
| prM/M | 834 | T | C | - | 44.44 | NS3 | 5844 | G | A | - | 11.76 |
| prM/M | 837 | T | C | - | 39.67 | NS3 | 5859 | T | C | - | 26.42 |
| prM/M | 852 | G | A | - | 39.45 | NS3 | 6009 | T | C | - | 33.87 |
| prM/M | 856 | C | T | - | 38.79 | NS3 | 6033 | C | T | - | 42.86 |
| prM/M | 864 | C | T | - | 29.60 | NS3 | 6069 | G | T | - | 33.33 |
| prM/M | 873 | A | C | - | 26.72 | NS3 | 6094 | T | C | - | 17.19 |
| prM/M | 885 | C | T | - | 8.09 | NS3 | 6157 | C | T | - | 29.69 |
| prM/M | 894 | C | A | - | 26.83 | NS3 | 6159 | A | G | - | 42.86 |
| prM/M | 921 | C | T | - | 19.08 | NS3 | 6300 | C | T | - | 26.56 |
| E | 954 | A | C | - | 14.75 | NS4A | 6378 | T | C | - | 33.33 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1005 | C | A | - | 30.40 | NS4A | 6379 | T | C | - | 34.00 |
| E | 1023 | T | C | - | 38.28 | NS4A | 6462 | T | C | - | 26.50 |
| E | 1094 | C | T | Pro53Leu | 21.21 | NS4A | 6471 | T | C | - | 10.40 |
| E | 1102 | C | T | - | 38.24 | NS4A | 6491 | A | G | Lys39Arg | 20.00 |
| E | 1214 | A | G | Lys93Arg | 18.42 | NS4A | 6496 | T | C | Tyr41His | 17.74 |
| E | 1287 | T | C | - | 11.11 | NS4A | 6500 | C | A | Thr42Asn | 16.54 |
| E | 1293 | C | T | - | 20.78 | NS4A | 6512 | A | G | Asn46Ser | 45.38 |
| E | 1302 | A | G | - | 8.86 | NS4A | 6528 | T | C | - | 30.30 |
| E | 1353 | C | T | - | 10.71 | NS4A | 6547 | C | T | - | 26.62 |
| E | 1396 | G | A | Asp154Asn | 12.73 | NS4A | 6556 | C | T | - | 24.81 |
| E | 1413 | C | A | - | 24.44 | NS4A | 6562 | G | A | Ala63Thr | 27.91 |
| E | 1473 | T | C | - | 25.42 | NS4A | 6585 | A | G | - | 21.60 |
| E | 1545 | C | T | - | 43.97 | NS4A | 6633 | T | C | - | 44.90 |
| E | 1590 | G | A | - | 29.09 | NS4A | 6654 | T | C | - | 41.57 |
| E | 1599 | G | A | - | 43.93 | NS4A | 6681 | A | G | - | 32.94 |
| E | 1626 | C | T | - | 47.92 | NS4A | 6727 | T | C | - | 12.82 |
| E | 1641 | G | A | - | 43.01 | NS4B | 6841 | C | T | - | 29.59 |
| E | 1647 | G | A | - | 8.70 | NS4B | 6864 | C | T | - | 13.76 |
| E | 1689 | T | C | - | 14.08 | NS4B | 6871 | G | A | Gly16Arg | 12.50 |
| E | 1842 | T | C | - | 20.75 | NS4B | 6879 | T | C | - | 35.71 |
| E | 1863 | G | A | - | 37.14 | NS4B | 6948 | T | C | - | 39.05 |
| E | 1911 | A | G | - | 10.64 | NS4B | 6951 | C | A | - | 8.19 |
| E | 1920 | G | T | - | 18.52 | NS4B | 6957 | T | C | - | 41.52 |
| E | 1944 | T | C | - | 45.71 | NS4B | 7008 | A | G | - | 48.30 |
| E | 1956 | A | T | - | 14.29 | NS4B | 7018 | C | T | - | 39.01 |
| E | 1959 | T | C | - | 27.78 | NS4B | 7038 | A | G | - | 15.47 |
| E | 1972 | C | T | His346Tyr | 25.33 | NS4B | 7041 | T | C | - | 44.20 |
| E | 2055 | C | T | - | 11.11 | NS4B | 7059 | T | C | - | 23.53 |
| E | 2142 | T | C | - | 7.27 | NS4B | 7062 | G | A | - | 42.01 |
| E | 2223 | T | C | - | 26.50 | NS4B | 7137 | T | C | - | 20.19 |
| E | 2241 | T | C | - | 6.20 | NS4B | 7165 | T | C | - | 41.67 |
| E | 2247 | T | C | - | 39.68 | NS4B | 7186 | A | G | ile121Val | 40.00 |
| E | 2256 | C | T | - | 8.59 | NS4B | 7248 | C | T | - | 26.03 |
| E | 2276 | C | T | Ala447Val | 11.47 | NS4B | 7314 | A | G | - | 42.39 |
| E | 2289 | C | T | - | 31.25 | NS4B | 7356 | C | T | - | 42.86 |
| E | 2307 | C | T | - | 30.50 | NS4B | 7383 | T | C | - | 41.90 |
| E | 2319 | C | A | - | 19.31 | NS4B | 7410 | C | T | - | 35.16 |
| E | 2376 | A | G | - | 28.81 | NS4B | 7428 | C | T | - | 39.78 |
| NS1 | 2434 | A | G | Ile5Val | 41.58 | NS4B | 7449 | T | C | - | 18.18 |
| NS1 | 2448 | A | G | - | 28.57 | NS5 | 7656 | G | A | - | 14.29 |
| NS1 | 2451 | T | C | - | 43.96 | NS5 | 7719 | G | A | - | 16.67 |
| NS1 | 2484 | T | C | - | 39.09 | NS5 | 7758 | A | G | - | 24.24 |
| NS1 | 2595 | C | T | - | 9.90 | NS5 | 7794 | G | A | - | 28.13 |
| NS1 | 2685 | T | C | - | 28.24 | NS5 | 7812 | T | C | - | 44.12 |
| NS1 | 2697 | C | T | - | 15.85 | NS5 | 7999 | T | C | - | 17.27 |
| NS1 | 2731 | T | C | - | 19.12 | NS5 | 8049 | A | G | - | 42.98 |
| NS1 | 2791 | C | T | Leu124Phe | 13.58 | NS5 | 8083 | C | T | - | 43.36 |
| NS1 | 2808 | C | T | - | 4.82 | NS5 | 8121 | T | C | - | 38.98 |
| NS1 | 2898 | T | C | - | 43.75 | NS5 | 8139 | C | T | - | 10.58 |
| NS1 | 2911 | A | T | Thr164Ser | 13.04 | NS5 | 8184 | A | G | - | 35.11 |
| NS1 | 2946 | G | A | - | 7.89 | NS5 | 8211 | C | T | - | 6.25 |
| NS1 | 2961 | C | T | - | 35.29 | NS5 | 8214 | G | A | - | 12.63 |
| NS1 | 2976 | A | G | - | 36.07 | NS5 | 8235 | T | C | - | 12.37 |
| NS1 | 2989 | G | A | Asp190Asn | 28.17 | NS5 | 8241 | T | C | - | 28.89 |
| NS1 | 2991 | C | T | - | 20.51 | NS5 | 8284 | T | C | - | 39.19 |
| NS1 | 3009 | C | T | - | 32.14 | NS5 | 8289 | T | C | - | 8.00 |
| NS1 | 3075 | T | C | - | 41.18 | NS5 | 8298 | C | T | - | 22.73 |
| NS1 | 3081 | T | A | - | 42.71 | NS5 | 8306 | A | G | Lys246Arg | 11.29 |
| NS1 | 3114 | C | A | - | 13.04 | NS5 | 8310 | T | C | - | 40.35 |
| NS1 | 3129 | A | G | - | 16.95 | NS5 | 8322 | C | A | - | 11.76 |

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|---|---|---|---|---|---|
| NS1 | 3152 | C | CA | Asn246fs | 6.78 |
| NS1 | 3165 | C | T | - | 6.67 |
| NS1 | 3204 | T | C | - | 6.06 |
| NS1 | 3234 | T | C | - | 44.66 |
| NS1 | 3261 | C | T | - | 3.45 |
| NS1 | 3330 | C | T | - | 26.19 |
| NS1 | 3342 | C | T | - | 7.63 |
| NS1 | 3382 | C | T | - | 7.55 |
| NS2A | 3489 | G | A | - | 44.44 |
| NS2A | 3595 | T | C | Phe40Leu | 36.73 |
| NS2A | 3604 | T | C | - | 23.08 |
| NS2A | 3612 | T | A | - | 25.00 |
| NS2A | 3624 | T | C | - | 36.94 |
| NS2A | 3629 | G | A | Arg51Lys | 39.09 |
| NS2A | 3663 | T | C | - | 38.52 |
| NS2A | 3750 | A | G | - | 17.03 |
| NS2A | 3753 | C | T | - | 28.81 |
| NS2A | 3768 | C | T | - | 31.58 |
| NS2A | 3801 | C | T | - | 10.49 |
| NS2A | 3858 | A | G | - | 14.18 |
| NS2A | 3864 | G | A | - | 38.60 |
| NS2A | 3875 | C | T | Ala133Val | 37.16 |
| NS2A | 3876 | C | T | - | 6.02 |
| NS2A | 3879 | C | A | - | 36.13 |
| NS2A | 3910 | T | C | - | 11.99 |
| NS2A | 3921 | T | C | - | 44.70 |
| NS2A | 4011 | T | C | - | 42.06 |
| NS2A | 4034 | A | G | Gln186Arg | 22.91 |
| NS2A | 4063 | T | C | - | 15.90 |
| NS2A | 4110 | T | C | - | 25.23 |
| NS2B | 4206 | T | C | - | 44.17 |
| NS2B | 4248 | C | T | - | 5.97 |
| NS2B | 4249 | G | A | Val40Ile | 9.85 |
| NS2B | 4257 | C | T | - | 9.32 |
| NS2B | 4335 | A | G | - | 18.60 |
| NS2B | 4344 | C | T | - | 50.00 |
| NS2B | 4350 | T | C | - | 34.55 |
| NS2B | 4482 | G | A | - | 16.67 |
| NS2B | 4509 | G | T | - | 43.08 |
| NS5 | 8331 | A | G | - | 18.60 |
| NS5 | 8526 | T | C | - | 9.38 |
| NS5 | 8541 | G | A | - | 12.07 |
| NS5 | 8574 | C | T | - | 11.48 |
| NS5 | 8604 | C | T | - | 17.02 |
| NS5 | 8658 | T | C | - | 29.41 |
| NS5 | 8745 | A | G | - | 30.77 |
| NS5 | 8751 | T | C | - | 35.71 |
| NS5 | 8775 | A | G | - | 14.81 |
| NS5 | 8803 | G | A | Val412Ile | 47.73 |
| NS5 | 8859 | G | T | Arg430Ser | 18.64 |
| NS5 | 8979 | T | C | - | 24.49 |
| NS5 | 9012 | A | G | - | 42.37 |
| NS5 | 9066 | C | T | - | 40.43 |
| NS5 | 9079 | C | T | - | 44.68 |
| NS5 | 9120 | C | T | - | 26.67 |
| NS5 | 9384 | T | C | - | 50.00 |
| NS5 | 9399 | T | C | - | 40.91 |
| NS5 | 9402 | T | C | - | 41.67 |
| NS5 | 9424 | T | C | - | 31.25 |
| NS5 | 9501 | G | C | Gln644His | 38.33 |
| NS5 | 9576 | T | C | - | 31.00 |
| NS5 | 9628 | G | A | Val687Ile | 10.23 |
| NS5 | 9726 | AG | A | Asp720fs | 2.40 |
| NS5 | 9795 | G | A | - | 14.86 |
| NS5 | 9810 | T | C | - | 11.45 |
| NS5 | 9825 | T | C | - | 30.49 |
| NS5 | 9849 | A | G | - | 5.81 |
| NS5 | 9888 | A | G | - | 33.91 |
| NS5 | 9902 | C | G | Ala778Gly | 5.61 |
| NS5 | 9942 | T | C | - | 13.89 |
| NS5 | 10075 | G | A | Val836Ile | 42.02 |
| NS5 | 10083 | C | T | - | 30.65 |
| NS5 | 10089 | G | A | - | 8.87 |
| 3UTR | 10280 | A | G | - | 22.22 |
| 3UTR | 10389 | C | CA | - | 19.85 |
| 3UTR | 10389 | C | CAA | - | 3.68 |
| 3UTR | 10407 | T | C | - | 24.31 |
| 3UTR | 10443 | T | TA | - | 2.92 |

| **197** | | | | | |
|---|---|---|---|---|---|
| SD | | | | | |
| Primary | | | | | |
| 4.17E+00 | | | | | |
| 6 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 111 | A | G | - | 31.43 |
| C | 201 | T | C | - | 24.53 |
| C | 219 | C | T | - | 10.71 |
| C | 219 | C | A | - | 5.36 |
| C | 246 | T | G | - | 9.09 |
| C | 252 | G | A | - | 9.26 |
| C | 294 | A | G | - | 8.00 |
| C | 315 | G | A | - | 17.07 |
| C | 324 | A | G | - | 9.52 |
| C | 336 | T | C | - | 13.64 |
| C | 345 | G | C | - | 11.36 |
| C | 348 | C | T | - | 11.36 |
| C | 375 | C | T | - | 21.43 |
| NS3 | 4623 | T | C | - | 19.05 |
| NS3 | 4629 | T | C | - | 26.09 |
| NS3 | 4635 | C | T | - | 31.82 |
| NS3 | 4641 | T | C | - | 40.91 |
| NS3 | 4662 | T | C | - | 32.26 |
| NS3 | 4695 | G | A | - | 23.81 |
| NS3 | 4703 | G | A | Arg61Lys | 17.39 |
| NS3 | 4731 | A | G | - | 21.05 |
| NS3 | 4739 | A | G | Lys73Arg | 17.39 |
| NS3 | 4746 | T | C | - | 29.63 |
| NS3 | 4755 | A | G | - | 12.12 |
| NS3 | 4812 | C | T | - | 36.36 |
| NS3 | 4830 | A | G | - | 16.33 |
| NS3 | 4905 | G | A | - | 15.79 |
| NS3 | 4944 | C | T | - | 16.22 |
| NS3 | 4959 | T | C | - | 19.40 |
| NS3 | 4971 | C | T | - | 29.41 |
| NS3 | 4974 | T | C | - | 10.45 |
| NS3 | 4992 | G | A | - | 4.84 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 384 | T | C | - | 43.90 | NS3 | 4999 | G | A | Ala160Thr | 6.06 |
| C | 387 | G | A | - | 33.33 | NS3 | 5004 | C | T | - | 35.09 |
| C | 399 | A | T | - | 6.82 | NS3 | 5007 | A | G | - | 7.55 |
| C | 431 | C | T | Ala112Val | 46.78 | NS3 | 5025 | C | T | - | 10.00 |
| C | 432 | G | A | - | 10.00 | NS3 | 5055 | C | T | - | 6.78 |
| prM/M | 445 | T | C | - | 18.97 | NS3 | 5067 | T | C | - | 8.20 |
| prM/M | 480 | T | C | - | 9.23 | NS3 | 5070 | C | T | - | 30.51 |
| prM/M | 483 | T | C | - | 9.52 | NS3 | 5073 | A | G | - | 6.67 |
| prM/M | 489 | G | A | - | 10.34 | NS3 | 5076 | G | A | - | 6.25 |
| prM/M | 507 | T | C | - | 10.34 | NS3 | 5078 | A | G | Lys186Arg | 6.35 |
| prM/M | 508 | C | T | - | 25.00 | NS3 | 5082 | G | A | - | 32.81 |
| prM/M | 523 | A | G | Asn29Asp | 39.06 | NS3 | 5091 | C | T | - | 8.62 |
| prM/M | 525 | C | T | - | 37.10 | NS3 | 5103 | T | C | - | 10.77 |
| prM/M | 531 | C | T | - | 30.43 | NS3 | 5109 | G | A | - | 6.45 |
| prM/M | 531 | C | G | - | 8.70 | NS3 | 5112 | A | G | - | 8.33 |
| prM/M | 555 | A | G | Ile39Met | 7.29 | NS3 | 5121 | A | G | - | 7.27 |
| prM/M | 567 | G | A | - | 22.47 | NS3 | 5190 | C | T | - | 16.25 |
| prM/M | 568 | T | C | - | 5.49 | NS3 | 5256 | A | C | - | 13.04 |
| prM/M | 651 | G | A | - | 13.33 | NS3 | 5268 | C | T | - | 17.07 |
| prM/M | 792 | G | A | - | 35.59 | NS3 | 5277 | C | T | - | 48.50 |
| prM/M | 795 | T | C | - | 6.78 | NS3 | 5283 | A | G | - | 28.21 |
| prM/M | 798 | T | C | - | 37.29 | NS3 | 5286 | G | A | - | 12.20 |
| prM/M | 834 | T | C | - | 14.10 | NS3 | 5295 | C | T | - | 10.00 |
| prM/M | 837 | T | C | - | 20.27 | NS3 | 5296 | C | T | - | 10.87 |
| prM/M | 852 | G | A | - | 26.76 | NS3 | 5304 | T | C | - | 18.18 |
| prM/M | 856 | C | T | - | 26.76 | NS3 | 5319 | T | C | - | 22.00 |
| prM/M | 864 | C | T | - | 12.79 | NS3 | 5340 | C | T | - | 20.00 |
| prM/M | 873 | A | C | - | 13.10 | NS3 | 5346 | G | A | - | 10.00 |
| prM/M | 876 | G | A | - | 8.45 | NS3 | 5364 | T | C | - | 20.00 |
| prM/M | 880 | C | T | His148Tyr | 7.79 | NS3 | 5367 | C | T | - | 17.95 |
| prM/M | 882 | C | T | - | 7.23 | NS3 | 5373 | C | T | - | 13.89 |
| prM/M | 891 | G | A | - | 6.74 | NS3 | 5451 | A | C | - | 30.00 |
| prM/M | 893 | C | T | Ala152Val | 6.90 | NS3 | 5460 | C | T | - | 28.57 |
| prM/M | 894 | C | A | - | 13.79 | NS3 | 5574 | C | T | - | 18.52 |
| prM/M | 921 | C | T | - | 19.75 | NS3 | 5583 | T | C | - | 27.59 |
| E | 960 | T | C | - | 9.20 | NS3 | 5610 | A | G | - | 20.83 |
| E | 969 | C | T | - | 11.36 | NS3 | 5613 | T | C | - | 27.27 |
| E | 972 | A | G | - | 11.11 | NS3 | 5631 | C | T | - | 21.05 |
| E | 1005 | C | A | - | 26.15 | NS3 | 5766 | C | T | - | 10.26 |
| E | 1023 | C | T | - | 21.92 | NS3 | 5781 | G | A | - | 15.38 |
| E | 1032 | G | A | - | 11.39 | NS3 | 5796 | T | C | - | 13.33 |
| E | 1039 | G | T | Ala35Ser | 5.56 | NS3 | 5799 | A | G | - | 13.33 |
| E | 1062 | T | C | - | 11.67 | NS3 | 5802 | C | T | - | 35.14 |
| E | 1068 | A | G | - | 13.46 | NS3 | 5817 | T | C | - | 11.90 |
| E | 1089 | A | G | - | 12.12 | NS3 | 5823 | A | G | - | 14.63 |
| E | 1102 | C | T | - | 18.18 | NS3 | 5847 | C | T | - | 42.42 |
| E | 1122 | A | G | - | 15.00 | NS3 | 5859 | T | C | - | 39.39 |
| E | 1148 | A | C | Glu71Ala | 19.05 | NS3 | 6009 | T | C | - | 45.16 |
| E | 1152 | G | T | - | 19.05 | NS3 | 6033 | T | C | - | 31.58 |
| E | 1155 | T | A | - | 19.05 | NS3 | 6048 | C | T | - | 9.09 |
| E | 1185 | T | C | - | 25.00 | NS3 | 6063 | A | G | - | 18.42 |
| E | 1207 | A | G | Ile91Val | 25.00 | NS3 | 6066 | A | G | - | 17.50 |
| E | 1218 | C | T | - | 16.67 | NS3 | 6069 | G | T | - | 9.52 |
| E | 1221 | C | T | - | 14.81 | NS3 | 6087 | A | G | - | 26.09 |
| E | 1227 | A | G | - | 16.13 | NS3 | 6093 | T | C | - | 27.91 |
| E | 1293 | T | C | - | 36.11 | NS3 | 6096 | G | A | - | 28.57 |
| E | 1396 | G | A | Asp154Asn | 18.18 | NS3 | 6108 | A | G | - | 31.91 |
| E | 1413 | C | A | - | 20.00 | NS3 | 6141 | G | A | - | 34.04 |
| E | 1473 | C | T | - | 47.06 | NS3 | 6157 | C | T | - | 27.03 |
| E | 1476 | T | C | - | 13.89 | NS3 | 6159 | G | A | - | 37.21 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1543 | G | A | Asp203Asn | 5.88 | NS3 | 6167 | A | G | Lys549Arg | 22.50 |
| E | 1545 | T | C | - | 36.76 | NS3 | 6177 | T | C | - | 19.51 |
| E | 1576 | C | T | - | 7.46 | NS3 | 6186 | C | T | - | 13.64 |
| E | 1590 | A | G | - | 46.15 | NS3 | 6192 | C | T | - | 13.04 |
| E | 1590 | G | A | - | 21.54 | NS3 | 6203 | A | G | Lys588Arg | 9.76 |
| E | 1599 | G | A | - | 26.67 | NS3 | 6220 | A | G | Ile567Val | 32.35 |
| E | 1626 | C | T | - | 31.75 | NS3 | 6222 | T | C | - | 22.86 |
| E | 1641 | G | A | - | 20.34 | NS3 | 6237 | A | C | - | 19.51 |
| E | 1713 | C | T | - | 6.25 | NS3 | 6238 | C | T | - | 17.07 |
| E | 1722 | G | A | - | 6.82 | NS3 | 6279 | G | A | - | 17.14 |
| E | 1740 | A | G | - | 7.14 | NS3 | 6288 | A | G | - | 17.65 |
| E | 1776 | G | A | - | 12.50 | NS3 | 6294 | A | G | - | 18.18 |
| E | 1858 | A | G | Ile308Val | 18.52 | NS3 | 6300 | C | T | - | 20.59 |
| E | 1860 | C | T | - | 18.52 | NS3 | 6303 | A | G | - | 15.15 |
| E | 1863 | G | A | - | 17.24 | NS3 | 6307 | T | C | - | 14.71 |
| E | 1944 | C | T | - | 37.50 | NS3 | 6312 | T | C | - | 17.50 |
| E | 1959 | C | T | - | 42.42 | NS3 | 6333 | A | G | - | 14.81 |
| E | 1972 | C | T | His346Tyr | 44.74 | NS3 | 6339 | A | G | - | 15.25 |
| E | 1995 | A | G | - | 32.65 | NS3 | 6342 | A | C | - | 14.29 |
| E | 2023 | A | G | Ser363Gly | 34.38 | NS3 | 6354 | G | A | - | 14.75 |
| E | 2024 | G | C | | 34.38 | NS3 | 6357 | A | G | - | 14.29 |
| E | 2064 | C | T | - | 19.35 | NS3 | 6366 | T | C | - | 13.56 |
| E | 2067 | T | C | - | 16.13 | NS4A | 6378 | T | C | - | 34.69 |
| E | 2073 | T | C | - | 16.13 | NS4A | 6379 | T | C | - | 34.69 |
| E | 2082 | A | G | - | 14.29 | NS4A | 6390 | C | T | - | 7.69 |
| E | 2142 | T | C | - | 22.22 | NS4A | 6423 | C | T | - | 9.09 |
| E | 2163 | A | G | - | 22.22 | NS4A | 6429 | T | C | - | 8.00 |
| E | 2175 | C | T | - | 22.73 | NS4A | 6435 | G | A | - | 6.85 |
| E | 2223 | T | C | - | 34.92 | NS4A | 6441 | A | G | - | 6.94 |
| E | 2241 | T | C | - | 19.35 | NS4A | 6444 | C | T | - | 7.35 |
| E | 2247 | T | C | - | 32.79 | NS4A | 6448 | C | T | - | 5.95 |
| E | 2250 | A | G | - | 16.39 | NS4A | 6462 | T | C | - | 13.16 |
| E | 2256 | C | T | - | 19.67 | NS4A | 6466 | C | T | - | 5.19 |
| E | 2277 | T | C | - | 10.17 | NS4A | 6491 | A | G | Lys39Arg | 8.11 |
| E | 2280 | T | C | - | 10.17 | NS4A | 6496 | T | C | Tyr41His | 8.54 |
| E | 2283 | T | C | - | 9.84 | NS4A | 6500 | C | A | Thr42Asn | 7.78 |
| E | 2289 | C | T | - | 18.03 | NS4A | 6512 | A | G | Asn46Ser | 23.29 |
| E | 2298 | T | C | - | 15.25 | NS4A | 6519 | G | C | - | 5.13 |
| E | 2307 | C | T | - | 26.87 | NS4A | 6528 | T | C | - | 15.85 |
| E | 2319 | C | A | - | 15.49 | NS4A | 6547 | C | T | - | 12.66 |
| E | 2319 | C | T | - | 9.86 | NS4A | 6556 | C | T | - | 12.05 |
| E | 2320 | A | G | Ile462Val | 9.59 | NS4A | 6562 | G | A | Ala63Thr | 18.52 |
| E | 2349 | T | C | - | 11.59 | NS4A | 6633 | T | C | - | 12.50 |
| E | 2376 | G | A | - | 36.67 | NS4A | 6654 | T | C | - | 15.87 |
| NS1 | 2434 | A | G | Ile5Val | 30.36 | NS4A | 6681 | A | G | - | 24.59 |
| NS1 | 2451 | T | C | - | 40.00 | NS4A | 6732 | C | T | - | 15.00 |
| NS1 | 2481 | C | T | - | 6.25 | NS4A | 6777 | G | A | - | 19.57 |
| NS1 | 2484 | T | C | - | 22.95 | NS4A | 6780 | C | T | - | 21.74 |
| NS1 | 2493 | C | T | - | 6.15 | NS4B | 6837 | T | G | - | 22.41 |
| NS1 | 2511 | G | A | - | 22.22 | NS4B | 6840 | C | T | - | 23.21 |
| NS1 | 2580 | C | T | - | 9.76 | NS4B | 6841 | C | T | - | 12.73 |
| NS1 | 2589 | A | G | - | 30.77 | NS4B | 6852 | C | G | - | 20.97 |
| NS1 | 2685 | T | C | - | 12.24 | NS4B | 6861 | C | T | - | 21.21 |
| NS1 | 2697 | C | T | - | 19.05 | NS4B | 6862 | C | T | Leu13Phe | 21.21 |
| NS1 | 2781 | G | A | - | 37.84 | NS4B | 6870 | T | G | Phe15Leu | 22.22 |
| NS1 | 2808 | C | T | - | 13.64 | NS4B | 6879 | T | C | - | 18.33 |
| NS1 | 2835 | T | C | - | 10.20 | NS4B | 6880 | A | G | Thr19Ala | 21.31 |
| NS1 | 2850 | C | T | - | 9.26 | NS4B | 6889 | G | C | Glu22Gln | 11.76 |
| NS1 | 2878 | C | A | Leu153Met | 42.50 | NS4B | 6891 | A | G | - | 11.76 |
| NS1 | 2880 | G | A | - | 7.32 | NS4B | 6892 | T | C | Ser23Pro | 12.12 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS1 | 2898 | T | C | - | 46.34 | NS4B | 6894 | T | C | - | 11.94 |
| NS1 | 2911 | A | T | Thr164Ser | 9.30 | NS4B | 6924 | T | C | - | 15.24 |
| NS1 | 2952 | A | G | - | 12.24 | NS4B | 6942 | G | C | - | 15.04 |
| NS1 | 2961 | C | T | - | 18.37 | NS4B | 6948 | T | C | - | 20.33 |
| NS1 | 2976 | A | G | - | 21.05 | NS4B | 6957 | C | T | - | 27.50 |
| NS1 | 2989 | A | G | Asn190Asp | 34.38 | NS4B | 6967 | G | A | Val48Ile | 15.56 |
| NS1 | 3009 | T | C | - | 40.32 | NS4B | 6979 | T | C | - | 13.25 |
| NS1 | 3057 | G | A | Met212Ile | 7.14 | NS4B | 6982 | C | A | - | 13.99 |
| NS1 | 3060 | G | A | - | 7.02 | NS4B | 6990 | C | T | - | 12.68 |
| NS1 | 3075 | T | C | - | 33.33 | NS4B | 7008 | A | G | - | 28.91 |
| NS1 | 3081 | T | A | - | 40.00 | NS4B | 7014 | C | G | - | 8.53 |
| NS1 | 3102 | G | A | - | 9.80 | NS4B | 7017 | C | T | - | 9.32 |
| NS1 | 3111 | C | T | - | 6.38 | NS4B | 7018 | T | C | - | 35.90 |
| NS1 | 3129 | A | G | - | 18.18 | NS4B | 7032 | T | C | - | 12.50 |
| NS1 | 3130 | T | C | - | 11.36 | NS4B | 7038 | A | G | - | 7.77 |
| NS1 | 3152 | C | CA | Asn246fs | 5.45 | NS4B | 7041 | T | C | - | 36.19 |
| NS1 | 3156 | A | G | - | 29.09 | NS4B | 7059 | T | C | - | 16.85 |
| NS1 | 3165 | C | T | - | 25.49 | NS4B | 7062 | G | A | - | 25.81 |
| NS1 | 3168 | G | A | - | 24.53 | NS4B | 7083 | G | A | - | 13.68 |
| NS1 | 3174 | G | A | - | 23.33 | NS4B | 7089 | C | T | - | 11.70 |
| NS1 | 3186 | C | T | - | 15.15 | NS4B | 7092 | C | T | - | 11.46 |
| NS1 | 3201 | C | T | - | 13.24 | NS4B | 7116 | A | G | - | 7.50 |
| NS1 | 3204 | T | C | - | 12.86 | NS4B | 7122 | C | T | - | 7.50 |
| NS1 | 3213 | A | G | - | 15.15 | NS4B | 7134 | C | T | - | 9.72 |
| NS1 | 3222 | T | C | - | 14.93 | NS4B | 7137 | T | C | - | 26.56 |
| NS1 | 3234 | T | C | - | 40.63 | NS4B | 7143 | C | T | - | 10.00 |
| NS1 | 3258 | C | T | - | 11.69 | NS4B | 7146 | C | T | - | 10.71 |
| NS1 | 3261 | C | T | - | 11.69 | NS4B | 7165 | T | C | - | 10.20 |
| NS1 | 3270 | T | C | - | 11.34 | NS4B | 7186 | G | A | Val121Ile | 24.14 |
| NS1 | 3289 | G | A | Asp290Asn | 10.53 | NS4B | 7200 | T | C | - | 25.00 |
| NS1 | 3313 | T | C | - | 12.90 | NS4B | 7206 | A | G | - | 20.83 |
| NS1 | 3324 | C | T | - | 12.64 | NS4B | 7248 | C | T | - | 19.23 |
| NS1 | 3327 | T | C | - | 12.64 | NS4B | 7314 | A | G | - | 22.03 |
| NS1 | 3330 | C | T | - | 22.73 | NS4B | 7356 | C | T | - | 32.73 |
| NS1 | 3339 | G | A | - | 13.00 | NS4B | 7383 | T | C | - | 35.59 |
| NS1 | 3357 | C | T | - | 14.68 | NS4B | 7410 | T | C | - | 36.67 |
| NS1 | 3366 | C | T | - | 12.63 | NS4B | 7428 | C | T | - | 35.00 |
| NS1 | 3373 | C | T | - | 12.77 | NS4B | 7435 | C | T | - | 8.62 |
| NS1 | 3381 | T | G | - | 9.64 | NS4B | 7497 | C | T | - | 13.33 |
| NS1 | 3402 | T | C | - | 22.78 | NS4B | 7509 | C | T | - | 15.38 |
| NS1 | 3408 | C | T | - | 13.04 | NS4B | 7518 | T | C | - | 26.09 |
| NS1 | 3417 | G | A | - | 11.11 | NS4B | 7557 | A | C | - | 13.64 |
| NS1 | 3432 | T | C | - | 41.51 | NS5 | 7686 | C | T | - | 25.93 |
| NS1 | 3432 | T | A | - | 9.43 | NS5 | 7777 | A | C | Met70Leu | 26.67 |
| NS1 | 3438 | A | G | - | 38.30 | NS5 | 7788 | G | A | - | 29.41 |
| NS1 | 3447 | A | G | - | 36.36 | NS5 | 7797 | G | A | - | 29.41 |
| NS1 | 3474 | A | G | - | 30.77 | NS5 | 7806 | T | C | - | 23.53 |
| NS2A | 3597 | T | C | - | 41.03 | NS5 | 7809 | T | C | - | 26.67 |
| NS2A | 3604 | T | C | - | 35.14 | NS5 | 7812 | T | C | - | 37.50 |
| NS2A | 3624 | T | C | - | 27.78 | NS5 | 7848 | A | C | - | 22.73 |
| NS2A | 3629 | G | A | Arg51Lys | 33.90 | NS5 | 7890 | A | G | - | 19.23 |
| NS2A | 3663 | C | T | - | 30.65 | NS5 | 7899 | C | T | - | 16.36 |
| NS2A | 3768 | C | T | - | 16.89 | NS5 | 7908 | C | T | - | 9.43 |
| NS2A | 3787 | G | A | Ala104Thr | 4.64 | NS5 | 7911 | C | T | - | 28.00 |
| NS2A | 3801 | C | T | - | 7.33 | NS5 | 7914 | C | A | - | 12.24 |
| NS2A | 3803 | C | T | Ala109Val | 5.81 | NS5 | 7947 | G | T | - | 6.67 |
| NS2A | 3807 | C | T | - | 5.52 | NS5 | 7956 | G | A | - | 7.89 |
| NS2A | 3816 | A | G | - | 5.10 | NS5 | 7959 | C | T | - | 30.00 |
| NS2A | 3825 | G | A | - | 3.29 | NS5 | 7971 | C | T | - | 8.26 |
| NS2A | 3844 | C | T | - | 4.19 | NS5 | 7999 | T | C | - | 15.09 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2A | 3864 | G | A | - | 29.05 | NS5 | 8035 | A | G | Ile156Val | 5.32 |
| NS2A | 3875 | C | T | Ala133Val | 27.52 | NS5 | 8037 | A | T | - | 5.49 |
| NS2A | 3876 | C | T | - | 9.09 | NS5 | 8049 | A | G | - | 21.00 |
| NS2A | 3879 | C | A | - | 20.37 | NS5 | 8064 | C | A | - | 7.32 |
| NS2A | 3900 | A | G | - | 6.92 | NS5 | 8070 | A | G | - | 10.10 |
| NS2A | 3903 | A | G | - | 6.14 | NS5 | 8083 | C | T | - | 15.46 |
| NS2A | 3906 | C | T | - | 5.78 | NS5 | 8088 | C | T | - | 8.57 |
| NS2A | 3912 | G | A | - | 8.89 | NS5 | 8091 | T | C | - | 9.62 |
| NS2A | 3921 | T | C | - | 22.40 | NS5 | 8115 | C | T | - | 10.31 |
| NS2A | 3935 | C | T | Ser153Leu | 8.95 | NS5 | 8121 | T | C | - | 22.22 |
| NS2A | 3936 | G | A | - | 8.85 | NS5 | 8127 | T | C | - | 7.29 |
| NS2A | 3942 | C | T | - | 9.18 | NS5 | 8143 | G | GA | Met194fs | 2.90 |
| NS2A | 3958 | T | C | - | 11.48 | NS5 | 8155 | A | G | Thr196Ala | 7.58 |
| NS2A | 3960 | G | A | - | 11.88 | NS5 | 8157 | A | G | - | 7.58 |
| NS2A | 3966 | C | T | - | 12.81 | NS5 | 8160 | A | G | - | 7.46 |
| NS2A | 3975 | G | A | - | 13.85 | NS5 | 8172 | T | C | - | 9.38 |
| NS2A | 3981 | T | C | - | 13.51 | NS5 | 8184 | A | G | - | 13.51 |
| NS2A | 3991 | T | C | - | 13.85 | NS5 | 8208 | C | T | - | 5.33 |
| NS2A | 3998 | C | T | Ala174Val | 15.15 | NS5 | 8214 | G | A | - | 5.63 |
| NS2A | 4009 | T | A | Ser178Thr | 13.07 | NS5 | 8226 | C | T | - | 8.57 |
| NS2A | 4011 | T | C | - | 32.21 | NS5 | 8235 | T | C | - | 11.94 |
| NS2A | 4021 | C | T | - | 16.96 | NS5 | 8241 | T | C | - | 22.58 |
| NS2A | 4034 | A | G | Gln186Arg | 29.73 | NS5 | 8242 | A | T | Thr225Ser | 11.29 |
| NS2A | 4047 | T | C | - | 15.79 | NS5 | 8284 | T | C | - | 31.48 |
| NS2A | 4057 | C | T | - | 14.56 | NS5 | 8289 | T | C | - | 22.22 |
| NS2A | 4063 | T | C | - | 8.15 | NS5 | 8298 | C | T | - | 14.63 |
| NS2A | 4071 | A | C | - | 15.60 | NS5 | 8306 | A | G | Lys246Arg | 30.00 |
| NS2A | 4074 | A | G | - | 15.94 | NS5 | 8310 | T | C | - | 27.27 |
| NS2A | 4083 | C | T | - | 34.90 | NS5 | 8316 | A | G | - | 24.14 |
| NS2A | 4098 | T | C | - | 15.22 | NS5 | 8322 | C | A | - | 19.35 |
| NS2A | 4107 | T | C | - | 13.61 | NS5 | 8337 | T | G | - | 15.00 |
| NS2A | 4110 | T | C | - | 13.57 | NS5 | 8469 | C | T | - | 35.71 |
| NS2A | 4113 | G | A | - | 13.04 | NS5 | 8526 | T | C | - | 8.89 |
| NS2A | 4121 | G | A | Ser215Asn | 13.28 | NS5 | 8535 | C | T | - | 8.70 |
| NS2B | 4134 | C | T | - | 14.50 | NS5 | 8541 | G | A | - | 12.77 |
| NS2B | 4140 | G | A | - | 14.53 | NS5 | 8544 | T | C | - | 40.43 |
| NS2B | 4149 | A | G | - | 15.25 | NS5 | 8572 | G | A | Val335Ile | 21.21 |
| NS2B | 4164 | C | T | - | 21.74 | NS5 | 8577 | T | C | - | 25.00 |
| NS2B | 4197 | A | G | - | 11.02 | NS5 | 8610 | T | C | - | 21.05 |
| NS2B | 4206 | T | C | - | 31.36 | NS5 | 8628 | T | C | - | 31.25 |
| NS2B | 4212 | T | C | - | 12.12 | NS5 | 8751 | T | C | - | 17.65 |
| NS2B | 4221 | T | A | - | 13.21 | NS5 | 8803 | G | A | Val412Ile | 36.84 |
| NS2B | 4225 | T | C | - | 14.55 | NS5 | 8823 | A | G | - | 17.86 |
| NS2B | 4242 | C | T | - | 14.68 | NS5 | 8829 | A | G | - | 13.33 |
| NS2B | 4248 | C | T | - | 13.68 | NS5 | 8832 | G | T | - | 11.76 |
| NS2B | 4251 | A | G | - | 15.96 | NS5 | 8859 | G | T | Arg430Ser | 20.00 |
| NS2B | 4270 | C | A | - | 13.64 | NS5 | 8967 | C | T | - | 30.56 |
| NS2B | 4278 | C | T | - | 36.14 | NS5 | 8979 | T | C | - | 42.42 |
| NS2B | 4305 | T | C | - | 16.92 | NS5 | 9012 | A | G | - | 46.43 |
| NS2B | 4310 | A | G | Lys60Arg | 18.33 | NS5 | 9018 | C | T | - | 18.75 |
| NS2B | 4323 | G | A | - | 15.22 | NS5 | 9042 | T | C | - | 16.13 |
| NS2B | 4329 | A | G | - | 41.03 | NS5 | 9066 | C | T | - | 25.00 |
| NS2B | 4338 | A | G | - | 13.89 | NS5 | 9399 | T | C | - | 45.45 |
| NS2B | 4341 | C | T | - | 24.24 | NS5 | 9501 | G | C | Gln644His | 11.90 |
| NS2B | 4344 | C | T | - | 39.39 | NS5 | 9576 | T | C | - | 8.51 |
| NS2B | 4368 | A | G | - | 20.59 | NS5 | 9595 | A | G | Ser676Gly | 7.41 |
| NS2B | 4386 | G | A | - | 30.00 | NS5 | 9600 | T | C | - | 8.20 |
| NS2B | 4401 | G | A | - | 26.32 | NS5 | 9606 | A | G | - | 7.84 |
| NS2B | 4414 | C | T | - | 26.32 | NS5 | 9708 | T | C | - | 5.00 |
| NS2B | 4434 | G | A | - | 33.33 | NS5 | 9744 | A | T | - | 5.75 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2B | 4441 | T | C | - | 23.53 | NS5 | 9747 | C | T | - | 5.49 |
| NS2B | 4449 | C | A | - | 19.05 | NS5 | 9795 | G | A | - | 17.81 |
| NS2B | 4456 | G | C | Val109Leu | 14.81 | NS5 | 9825 | T | C | - | 25.00 |
| NS2B | 4458 | C | T | - | 16.00 | NS5 | 9888 | G | A | - | 27.17 |
| NS2B | 4464 | A | C | - | 18.52 | NS5 | 9903 | C | T | - | 4.21 |
| NS2B | 4470 | G | A | - | 13.33 | NS5 | 9921 | G | A | - | 4.76 |
| NS2B | 4482 | G | A | - | 20.69 | NS5 | 9969 | G | A | - | 28.57 |
| NS2B | 4491 | A | G | - | 31.43 | NS5 | 9987 | G | A | - | 36.36 |
| NS2B | 4509 | G | T | - | 38.89 | NS5 | 10059 | G | A | - | 6.85 |
| NS2B | 4512 | G | A | - | 15.79 | NS5 | 10075 | A | G | Ile836Val | 29.69 |
| NS3 | 4539 | C | T | - | 15.79 | NS5 | 10083 | C | T | - | 18.52 |
| NS3 | 4542 | C | T | - | 16.28 | NS5 | 10155 | T | C | - | 27.45 |
| NS3 | 4557 | A | C | - | 15.79 | NS5 | 10202 | G | A | Gly878Glu | 40.91 |
| NS3 | 4566 | A | G | - | 19.44 | 3UTR | 10389 | C | CA | - | 5.21 |
| NS3 | 4596 | C | T | - | 46.15 | 3UTR | 10389 | C | CAA | - | 3.13 |
| NS3 | 4602 | A | G | - | 16.67 | 3UTR | 10407 | T | C | - | 9.71 |
| NS3 | 4604 | G | A | Arg28Lys | 16.67 | 3UTR | 10443 | T | TA | - | 2.56 |

| **198** | | | | | | NS3 | 5460 | T | C | - | 38.71 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SD | | | | | | NS3 | 5502 | G | A | - | 11.11 |
| Primary | | | | | | NS3 | 5568 | A | G | - | 24.39 |
| 3.79E+01 | | | | | | NS3 | 5577 | G | A | - | 25.00 |
| 7 | | | | | | NS3 | 5613 | T | C | - | 26.67 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5649 | G | A | - | 14.89 |
| C | 201 | T | C | - | 34.67 | NS3 | 5667 | G | A | - | 16.67 |
| C | 231 | A | G | - | 7.35 | NS3 | 5730 | C | T | - | 18.75 |
| C | 393 | T | C | - | 12.50 | NS3 | 5751 | T | C | - | 14.71 |
| prM/M | 450 | T | C | - | 37.10 | NS3 | 5754 | A | G | - | 14.29 |
| prM/M | 531 | T | C | - | 5.63 | NS3 | 5835 | A | G | - | 43.75 |
| prM/M | 606 | C | T | - | 22.00 | NS3 | 5889 | A | G | - | 12.12 |
| prM/M | 651 | G | A | - | 41.67 | NS3 | 6009 | T | C | - | 44.26 |
| prM/M | 885 | C | T | - | 13.33 | NS3 | 6063 | A | G | - | 6.52 |
| prM/M | 891 | G | A | - | 4.35 | NS3 | 6066 | A | G | - | 7.14 |
| prM/M | 893 | C | T | Ala152Val | 4.41 | NS3 | 6069 | G | T | - | 42.86 |
| prM/M | 894 | C | A | - | 45.59 | NS3 | 6094 | T | C | - | 41.30 |
| E | 954 | A | C | - | 38.89 | NS3 | 6099 | A | G | - | 42.55 |
| E | 969 | C | T | - | 5.88 | NS3 | 6220 | A | G | Ile567Val | 30.65 |
| E | 972 | A | G | - | 5.80 | NS3 | 6222 | T | C | - | 22.22 |
| E | 1005 | C | A | - | 44.62 | NS3 | 6300 | C | T | - | 45.00 |
| E | 1094 | C | T | Pro53Leu | 14.52 | NS3 | 6330 | C | T | - | 22.45 |
| E | 1102 | C | T | - | 32.76 | NS4A | 6378 | T | C | - | 45.65 |
| E | 1214 | A | G | Lys93Arg | 20.00 | NS4A | 6379 | T | C | - | 45.65 |
| E | 1287 | T | C | - | 9.52 | NS4A | 6444 | C | T | - | 17.86 |
| E | 1293 | T | C | - | 38.24 | NS4A | 6462 | T | C | - | 25.00 |
| E | 1413 | C | A | - | 33.33 | NS4A | 6471 | T | C | - | 40.26 |
| E | 1415 | A | G | Lys160Arg | 12.12 | NS4A | 6491 | A | G | Lys39Arg | 36.84 |
| E | 1543 | G | A | Asp203Asn | 16.39 | NS4A | 6496 | T | C | Tyr41His | 36.00 |
| E | 1689 | T | C | - | 21.57 | NS4A | 6500 | C | A | Thr42Asn | 33.75 |
| E | 1995 | A | G | - | 27.45 | NS4A | 6512 | G | A | Ser46Asn | 7.58 |
| E | 2007 | C | T | - | 11.43 | NS4A | 6528 | T | C | - | 42.86 |
| E | 2016 | A | G | - | 21.05 | NS4A | 6547 | C | T | - | 38.27 |
| E | 2064 | C | T | - | 30.77 | NS4A | 6556 | C | T | - | 36.59 |
| E | 2082 | A | G | - | 22.50 | NS4A | 6562 | G | A | Ala63Thr | 37.97 |
| E | 2105 | A | G | Asn390Ser | 18.92 | NS4A | 6585 | A | G | - | 39.08 |
| E | 2223 | C | T | - | 46.88 | NS4A | 6633 | T | C | - | 34.21 |
| E | 2256 | C | T | - | 45.33 | NS4A | 6654 | T | C | - | 41.18 |
| E | 2276 | C | T | Ala447Val | 9.20 | NS4A | 6727 | T | C | - | 14.29 |
| E | 2328 | A | T | - | 9.09 | NS4B | 6864 | C | T | - | 26.32 |
| NS1 | 2442 | C | T | - | 5.17 | NS4B | 6871 | G | A | Gly16Arg | 22.22 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS1 | 2448 | G | A | - | 48.15 | NS4B | 6879 | T | C | - | 38.71 |
| NS1 | 2511 | G | A | - | 46.58 | NS4B | 6948 | T | C | - | 49.45 |
| NS1 | 2589 | A | G | - | 42.19 | NS4B | 6951 | C | A | - | 16.84 |
| NS1 | 2685 | T | C | - | 15.07 | NS4B | 6957 | T | C | - | 6.19 |
| NS1 | 2731 | T | C | - | 30.19 | NS4B | 6990 | C | T | - | 3.62 |
| NS1 | 2772 | A | G | - | 9.86 | NS4B | 7008 | G | A | - | 16.54 |
| NS1 | 2791 | C | T | Leu124Phe | 20.00 | NS4B | 7038 | A | G | - | 9.91 |
| NS1 | 2808 | C | T | - | 7.14 | NS4B | 7083 | G | A | - | 5.06 |
| NS1 | 2878 | C | A | Leu153Met | 44.44 | NS4B | 7161 | T | C | - | 32.76 |
| NS1 | 2898 | T | C | - | 21.88 | NS4B | 7248 | C | T | - | 30.91 |
| NS1 | 2911 | A | T | Thr164Ser | 22.73 | NS4B | 7410 | C | T | - | 6.06 |
| NS1 | 2961 | C | T | - | 22.50 | NS4B | 7449 | T | C | - | 25.58 |
| NS1 | 2966 | A | G | Lys182Arg | 5.13 | NS5 | 7758 | A | G | - | 15.00 |
| NS1 | 2976 | A | G | - | 35.00 | NS5 | 7776 | C | T | - | 31.58 |
| NS1 | 2991 | C | T | - | 21.21 | NS5 | 7806 | C | T | - | 47.37 |
| NS1 | 3075 | T | C | - | 24.39 | NS5 | 7849 | C | T | - | 9.80 |
| NS1 | 3114 | A | C | - | 33.33 | NS5 | 7911 | C | T | - | 50.00 |
| NS1 | 3129 | A | G | - | 14.81 | NS5 | 7999 | T | C | - | 17.44 |
| NS1 | 3165 | C | T | - | 7.14 | NS5 | 8083 | C | T | - | 38.10 |
| NS1 | 3300 | T | C | - | 20.69 | NS5 | 8103 | T | C | - | 12.66 |
| NS1 | 3342 | C | T | - | 10.39 | NS5 | 8121 | T | C | - | 39.74 |
| NS1 | 3402 | T | C | - | 42.03 | NS5 | 8139 | C | T | - | 10.39 |
| NS2A | 3489 | G | A | - | 24.24 | NS5 | 8184 | A | G | - | 43.10 |
| NS2A | 3562 | A | G | Thr29Ala | 9.76 | NS5 | 8214 | G | A | - | 11.32 |
| NS2A | 3595 | T | C | Phe40Leu | 25.49 | NS5 | 8241 | T | C | - | 23.68 |
| NS2A | 3612 | T | A | - | 23.53 | NS5 | 8284 | T | C | - | 40.00 |
| NS2A | 3743 | C | T | Ala89Val | 25.66 | NS5 | 8289 | T | C | - | 33.96 |
| NS2A | 3750 | A | G | - | 14.05 | NS5 | 8298 | C | T | - | 43.14 |
| NS2A | 3855 | G | A | - | 4.55 | NS5 | 8310 | T | C | - | 14.63 |
| NS2A | 3858 | A | G | - | 12.21 | NS5 | 8380 | A | G | Ile271Val | 28.57 |
| NS2A | 3876 | C | T | - | 20.65 | NS5 | 8574 | C | T | - | 36.96 |
| NS2A | 3906 | C | T | - | 14.72 | NS5 | 8604 | C | T | - | 29.63 |
| NS2A | 4020 | C | T | - | 9.33 | NS5 | 8658 | T | C | - | 50.00 |
| NS2A | 4042 | G | T | Ala189Ser | 10.00 | NS5 | 8658 | C | T | - | 50.00 |
| NS2A | 4063 | T | C | - | 39.39 | NS5 | 8803 | G | A | Val412Ile | 40.00 |
| NS2A | 4110 | C | T | - | 31.20 | NS5 | 8823 | A | G | - | 23.68 |
| NS2B | 4206 | C | T | - | 29.32 | NS5 | 8967 | C | T | - | 48.15 |
| NS2B | 4338 | A | G | - | 45.45 | NS5 | 9042 | T | C | - | 21.95 |
| NS2B | 4344 | C | T | - | 39.13 | NS5 | 9066 | C | T | - | 33.33 |
| NS2B | 4350 | T | C | - | 34.88 | NS5 | 9079 | C | T | - | 26.47 |
| NS2B | 4374 | T | C | - | 10.53 | NS5 | 9112 | C | T | - | 38.10 |
| NS2B | 4512 | G | A | - | 8.70 | NS5 | 9132 | G | A | - | 41.67 |
| NS3 | 4596 | C | T | - | 34.48 | NS5 | 9384 | T | C | - | 40.00 |
| NS3 | 4629 | T | C | - | 12.12 | NS5 | 9399 | T | C | - | 33.33 |
| NS3 | 4806 | C | T | - | 33.33 | NS5 | 9402 | T | C | - | 33.33 |
| NS3 | 4854 | G | A | - | 14.75 | NS5 | 9424 | T | C | - | 34.78 |
| NS3 | 4878 | C | T | - | 10.14 | NS5 | 9491 | T | C | Ile641Thr | 22.86 |
| NS3 | 4944 | C | T | - | 36.84 | NS5 | 9501 | G | C | Gln644His | 31.82 |
| NS3 | 4956 | A | G | - | 10.00 | NS5 | 9576 | T | C | - | 22.69 |
| NS3 | 4959 | T | C | - | 41.67 | NS5 | 9628 | G | A | Val687Ile | 13.86 |
| NS3 | 4974 | T | C | - | 17.14 | NS5 | 9810 | T | C | - | 17.50 |
| NS3 | 5004 | C | T | - | 45.28 | NS5 | 9828 | T | C | - | 11.49 |
| NS3 | 5112 | A | G | - | 15.69 | NS5 | 9840 | T | C | - | 14.86 |
| NS3 | 5235 | T | C | - | 41.38 | NS5 | 9849 | A | G | - | 16.18 |
| NS3 | 5277 | C | T | - | 40.63 | NS5 | 10050 | A | G | - | 4.35 |
| NS3 | 5283 | A | G | - | 37.14 | NS5 | 10071 | A | T | Glu834Asp | 31.25 |
| NS3 | 5304 | T | C | - | 38.10 | 3UTR | 10389 | C | CA | - | 35.56 |
| NS3 | 5346 | G | A | - | 11.76 | 3UTR | 10389 | C | CAA | - | 11.11 |
| NS3 | 5451 | C | A | - | 42.86 | 3UTR | 10407 | C | T | - | 46.00 |

| 199 | | | | | |
|---|---|---|---|---|---|
| SD | | | | | |
| Secondary | | | | | |
| 2.25E+02 | | | | | |
| 7 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 201 | T | C | - | 46.00 |
| C | 312 | C | T | - | 15.79 |
| C | 315 | G | A | - | 36.84 |
| C | 327 | T | C | - | 26.67 |
| C | 431 | C | T | Ala112Val | 5.71 |
| prM/M | 450 | C | T | - | 38.60 |
| prM/M | 508 | C | T | - | 27.78 |
| prM/M | 531 | T | C | - | 8.97 |
| prM/M | 567 | G | A | - | 41.82 |
| prM/M | 606 | C | T | - | 30.61 |
| prM/M | 651 | G | A | - | 38.24 |
| prM/M | 717 | G | A | - | 41.18 |
| prM/M | 834 | T | C | - | 38.98 |
| prM/M | 837 | T | C | - | 38.60 |
| prM/M | 852 | G | A | - | 45.90 |
| prM/M | 856 | C | T | - | 43.55 |
| prM/M | 864 | C | T | - | 41.18 |
| prM/M | 873 | A | C | - | 40.68 |
| prM/M | 885 | C | T | - | 11.29 |
| prM/M | 894 | C | A | - | 30.77 |
| prM/M | 921 | T | C | - | 45.76 |
| E | 954 | A | C | - | 23.73 |
| E | 1005 | C | A | - | 41.18 |
| E | 1023 | T | C | - | 27.59 |
| E | 1040 | C | CA | Asn37fs | 7.55 |
| E | 1094 | C | T | Pro53Leu | 25.93 |
| E | 1102 | C | T | - | 43.33 |
| E | 1293 | T | C | - | 36.00 |
| E | 1353 | C | T | - | 5.88 |
| E | 1413 | C | A | - | 30.77 |
| E | 1479 | C | T | - | 12.96 |
| E | 1599 | A | G | - | 13.56 |
| E | 1641 | G | A | - | 33.33 |
| E | 1689 | T | C | - | 20.83 |
| E | 1863 | G | A | - | 27.59 |
| E | 1944 | T | C | - | 23.08 |
| E | 1959 | T | C | - | 17.95 |
| E | 1986 | C | T | - | 15.00 |
| E | 2007 | C | T | - | 16.67 |
| E | 2082 | A | G | - | 18.18 |
| E | 2223 | T | C | - | 42.42 |
| E | 2247 | T | C | - | 46.38 |
| E | 2256 | C | T | - | 22.54 |
| E | 2289 | C | T | - | 44.59 |
| E | 2307 | C | T | - | 37.33 |
| E | 2318 | T | C | Val461Ala | 12.50 |
| E | 2319 | C | A | - | 26.92 |
| E | 2328 | A | T | - | 24.10 |
| NS1 | 2448 | A | G | - | 46.00 |
| NS1 | 2511 | G | A | - | 49.12 |
| NS1 | 2589 | A | G | - | 39.13 |
| NS1 | 2685 | T | C | - | 11.29 |
| NS1 | 2731 | T | C | - | 12.24 |
| NS1 | 2781 | G | A | - | 35.71 |
| NS2B | 4443 | G | T | - | 7.50 |
| NS3 | 4707 | G | A | - | 14.71 |
| NS3 | 4806 | C | T | - | 21.05 |
| NS3 | 4854 | G | A | - | 20.41 |
| NS3 | 4905 | G | A | - | 40.38 |
| NS3 | 4944 | C | T | - | 42.22 |
| NS3 | 4959 | T | C | - | 42.50 |
| NS3 | 4971 | T | C | - | 17.65 |
| NS3 | 4974 | T | C | - | 18.00 |
| NS3 | 5070 | C | T | - | 41.27 |
| NS3 | 5190 | C | T | - | 32.20 |
| NS3 | 5235 | T | C | - | 25.42 |
| NS3 | 5304 | T | C | - | 43.33 |
| NS3 | 5502 | G | A | - | 17.86 |
| NS3 | 5730 | C | T | - | 10.81 |
| NS3 | 5751 | T | C | - | 21.05 |
| NS3 | 5754 | A | G | - | 21.05 |
| NS3 | 5835 | A | G | - | 33.33 |
| NS3 | 5847 | C | T | - | 41.38 |
| NS3 | 5859 | T | C | - | 16.67 |
| NS3 | 6069 | G | T | - | 41.67 |
| NS3 | 6157 | T | C | - | 23.26 |
| NS3 | 6159 | G | A | - | 15.91 |
| NS3 | 6220 | A | G | Ile567Val | 44.74 |
| NS3 | 6222 | T | C | - | 38.46 |
| NS3 | 6262 | A | G | Ile581Val | 12.50 |
| NS3 | 6300 | C | T | - | 33.33 |
| NS3 | 6330 | T | C | - | 36.36 |
| NS3 | 6351 | C | T | - | 19.05 |
| NS4A | 6378 | T | C | - | 35.00 |
| NS4A | 6379 | T | C | - | 34.43 |
| NS4A | 6462 | T | C | - | 32.73 |
| NS4A | 6471 | T | C | - | 36.67 |
| NS4A | 6491 | A | G | Lys39Arg | 38.71 |
| NS4A | 6496 | T | C | Tyr41His | 35.38 |
| NS4A | 6500 | C | A | Thr42Asn | 30.14 |
| NS4A | 6512 | G | A | Ser46Asn | 11.59 |
| NS4A | 6528 | T | C | - | 22.06 |
| NS4A | 6547 | C | T | - | 22.54 |
| NS4A | 6556 | C | T | - | 28.38 |
| NS4A | 6562 | G | A | Ala63Thr | 30.99 |
| NS4A | 6585 | G | A | - | 49.33 |
| NS4A | 6633 | T | C | - | 30.56 |
| NS4A | 6654 | T | C | - | 32.31 |
| NS4A | 6681 | A | G | - | 20.41 |
| NS4B | 6841 | C | T | - | 43.40 |
| NS4B | 6864 | C | T | - | 31.58 |
| NS4B | 6871 | G | A | Gly16Arg | 33.33 |
| NS4B | 6879 | T | C | - | 49.25 |
| NS4B | 6884 | C | T | Thr20Ile | 20.27 |
| NS4B | 6951 | C | A | - | 13.25 |
| NS4B | 6957 | T | C | - | 8.79 |
| NS4B | 7008 | G | A | - | 16.98 |
| NS4B | 7137 | T | C | - | 35.85 |
| NS4B | 7165 | T | C | - | 38.64 |
| NS4B | 7186 | A | G | ile121Val | 17.07 |
| NS4B | 7248 | C | T | - | 48.15 |
| NS4B | 7314 | A | G | - | 41.18 |
| NS4B | 7356 | C | T | - | 45.28 |
| NS4B | 7383 | T | C | - | 48.28 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS1 | 2791 | C | T | Leu124Phe | 11.67 | NS4B | 7410 | C | T | - | 13.56 |
| NS1 | 2878 | C | A | Leu153Met | 16.67 | NS4B | 7449 | C | T | - | 45.31 |
| NS1 | 2898 | T | C | - | 15.56 | NS4B | 7533 | C | T | - | 13.33 |
| NS1 | 2911 | A | T | Thr164Ser | 38.10 | NS5 | 7656 | G | A | - | 13.04 |
| NS1 | 2961 | C | T | - | 23.40 | NS5 | 7719 | G | A | - | 16.67 |
| NS1 | 2976 | A | G | - | 18.18 | NS5 | 7758 | A | G | - | 23.53 |
| NS1 | 2989 | G | A | Asp190Asn | 13.04 | NS5 | 7794 | G | A | - | 23.81 |
| NS1 | 2991 | C | T | - | 48.84 | NS5 | 7999 | T | C | - | 18.06 |
| NS1 | 3075 | T | C | - | 36.17 | NS5 | 8049 | A | G | - | 46.97 |
| NS1 | 3081 | T | A | - | 19.57 | NS5 | 8083 | C | T | - | 39.39 |
| NS1 | 3114 | C | A | - | 31.58 | NS5 | 8089 | A | G | Asn174Asp | 18.67 |
| NS1 | 3129 | A | G | - | 28.95 | NS5 | 8103 | T | C | - | 18.75 |
| NS1 | 3152 | C | CA | Asn246fs | 5.00 | NS5 | 8121 | T | C | - | 30.59 |
| NS1 | 3165 | C | T | - | 12.20 | NS5 | 8139 | C | T | - | 8.16 |
| NS1 | 3174 | G | A | - | 8.16 | NS5 | 8184 | A | G | - | 20.24 |
| NS1 | 3330 | C | T | - | 25.00 | NS5 | 8214 | G | A | - | 14.00 |
| NS1 | 3342 | C | T | - | 11.59 | NS5 | 8235 | T | C | - | 17.86 |
| NS2A | 3489 | G | A | - | 32.35 | NS5 | 8241 | T | C | - | 39.58 |
| NS2A | 3595 | T | C | Phe40Leu | 21.82 | NS5 | 8284 | T | C | - | 37.50 |
| NS2A | 3612 | T | A | - | 15.25 | NS5 | 8298 | C | T | - | 21.62 |
| NS2A | 3663 | T | C | - | 9.09 | NS5 | 8310 | T | C | - | 31.03 |
| NS2A | 3720 | C | T | - | 6.67 | NS5 | 8469 | C | T | - | 31.03 |
| NS2A | 3750 | A | G | - | 9.09 | NS5 | 8574 | C | T | - | 38.64 |
| NS2A | 3753 | C | T | - | 34.55 | NS5 | 8604 | C | T | - | 21.62 |
| NS2A | 3768 | C | T | - | 36.54 | NS5 | 8745 | A | G | - | 16.67 |
| NS2A | 3801 | C | T | - | 7.26 | NS5 | 8803 | G | A | Val412Ile | 27.27 |
| NS2A | 3858 | A | G | - | 18.40 | NS5 | 8823 | A | G | - | 24.32 |
| NS2A | 3864 | G | A | - | 46.40 | NS5 | 9042 | T | C | - | 23.53 |
| NS2A | 3875 | C | T | Ala133Val | 46.03 | NS5 | 9399 | T | C | - | 27.27 |
| NS2A | 3876 | C | T | - | 38.40 | NS5 | 9402 | T | C | - | 33.33 |
| NS2A | 3879 | C | A | - | 43.51 | NS5 | 9424 | T | C | - | 20.00 |
| NS2A | 3921 | T | C | - | 43.18 | NS5 | 9576 | T | C | - | 22.37 |
| NS2A | 4011 | T | C | - | 48.33 | NS5 | 9628 | G | A | Val687Ile | 26.15 |
| NS2A | 4034 | A | G | Gln186Arg | 41.35 | NS5 | 9810 | T | C | - | 16.88 |
| NS2A | 4047 | T | C | - | 6.19 | NS5 | 9840 | T | C | - | 20.25 |
| NS2A | 4057 | C | T | - | 5.50 | NS5 | 9849 | A | G | - | 20.25 |
| NS2A | 4063 | T | C | - | 28.42 | NS5 | 9942 | T | C | - | 15.79 |
| NS2A | 4098 | T | C | - | 6.38 | NS5 | 9969 | G | A | - | 21.43 |
| NS2A | 4110 | T | C | - | 31.87 | NS5 | 9984 | G | A | - | 45.45 |
| NS2B | 4164 | C | T | - | 44.44 | NS5 | 9987 | G | A | - | 45.45 |
| NS2B | 4206 | T | C | - | 42.17 | NS5 | 10000 | G | A | Ala811Thr | 27.27 |
| NS2B | 4257 | C | T | - | 6.49 | NS5 | 10075 | G | A | Val836Ile | 15.07 |
| NS2B | 4341 | C | T | - | 27.27 | NS5 | 10083 | C | T | - | 31.17 |
| NS2B | 4350 | C | T | - | 25.81 | NS5 | 10089 | G | A | - | 23.61 |
| NS2B | 4414 | C | T | - | 20.83 | 3UTR | 10407 | T | C | - | 34.62 |

| **200** | | | | | | NS3 | 4635 | C | T | - | 14.29 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SD | | | | | | NS3 | 4641 | T | C | - | 13.95 |
| Secondary | | | | | | NS3 | 4662 | T | C | - | 12.82 |
| 6.69E+02 | | | | | | NS3 | 4764 | A | G | - | 15.79 |
| 4 | | | | | | NS3 | 4800 | G | A | - | 6.85 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 4809 | A | G | - | 6.49 |
| C | 111 | A | G | - | 37.50 | NS3 | 4813 | C | T | - | 6.49 |
| C | 127 | A | T | Thr11Ser | 16.67 | NS3 | 4824 | A | G | - | 5.68 |
| C | 201 | T | C | - | 47.13 | NS3 | 4830 | A | G | - | 41.89 |
| C | 213 | A | G | - | 7.32 | NS3 | 4839 | C | A | - | 6.32 |
| C | 219 | C | T | - | 7.79 | NS3 | 4854 | G | A | - | 5.88 |
| C | 219 | C | A | - | 6.49 | NS3 | 4872 | A | G | - | 4.49 |
| C | 231 | A | G | - | 8.51 | NS3 | 4875 | C | T | - | 4.35 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 246 | T | G | - | 5.94 | NS3 | 4905 | G | A | - | 48.90 |
| C | 252 | G | A | - | 4.55 | NS3 | 4914 | C | T | - | 4.40 |
| C | 294 | A | G | - | 12.82 | NS3 | 4938 | T | C | - | 4.39 |
| C | 315 | G | A | - | 29.73 | NS3 | 4944 | C | T | - | 43.24 |
| C | 318 | A | G | - | 10.67 | NS3 | 4959 | T | C | - | 48.62 |
| C | 324 | A | G | - | 11.11 | NS3 | 4971 | T | C | - | 36.13 |
| C | 336 | T | C | - | 10.13 | NS3 | 4974 | T | C | - | 27.35 |
| C | 345 | G | C | - | 9.33 | NS3 | 4989 | A | T | - | 4.85 |
| C | 348 | C | T | - | 9.46 | NS3 | 4992 | G | A | - | 5.17 |
| C | 375 | C | T | - | 13.56 | NS3 | 5026 | G | GA | Ser171fs | 12.94 |
| C | 384 | T | C | - | 43.75 | NS3 | 5055 | C | T | - | 6.41 |
| C | 387 | G | A | - | 31.82 | NS3 | 5070 | C | T | - | 43.08 |
| C | 393 | T | C | - | 31.00 | NS3 | 5076 | G | A | - | 7.89 |
| C | 396 | G | A | - | 33.30 | NS3 | 5078 | A | G | Lys186Arg | 6.49 |
| C | 399 | A | T | - | 31.00 | NS3 | 5082 | A | G | - | 41.38 |
| C | 401 | C | T | Ala102Val | 31.00 | NS3 | 5091 | C | T | - | 10.53 |
| C | 406 | G | A | Val104Met | 21.90 | NS3 | 5103 | T | C | - | 10.34 |
| C | 411 | T | C | - | 14.71 | NS3 | 5109 | G | A | - | 9.09 |
| C | 412 | G | A | Val106Ile | 19.40 | NS3 | 5112 | A | G | - | 9.33 |
| C | 414 | T | C | - | 19.40 | NS3 | 5121 | A | G | - | 12.50 |
| C | 418 | T | C | - | 7.41 | NS3 | 5130 | C | T | - | 11.11 |
| C | 431 | C | T | Ala112Val | 30.00 | NS3 | 5139 | C | A | - | 11.25 |
| C | 432 | G | A | - | 6.45 | NS3 | 5145 | T | C | - | 9.57 |
| prM/M | 481 | G | A | Gly15Ser | 3.07 | NS3 | 5151 | A | G | - | 12.05 |
| prM/M | 486 | G | A | - | 3.16 | NS3 | 5163 | T | A | - | 9.38 |
| prM/M | 489 | G | A | - | 3.33 | NS3 | 5187 | T | C | - | 11.76 |
| prM/M | 507 | T | C | - | 3.70 | NS3 | 5190 | C | T | - | 29.52 |
| prM/M | 513 | C | T | - | 3.79 | NS3 | 5235 | T | C | - | 7.29 |
| prM/M | 523 | G | A | Asp29Asn | 35.61 | NS3 | 5256 | A | C | - | 10.84 |
| prM/M | 525 | T | C | - | 35.88 | NS3 | 5268 | C | T | - | 9.38 |
| prM/M | 531 | T | G | - | 4.90 | NS3 | 5295 | C | T | - | 6.76 |
| prM/M | 531 | T | C | - | 34.97 | NS3 | 5296 | C | T | - | 6.85 |
| prM/M | 555 | A | G | Ile39Met | 3.57 | NS3 | 5319 | T | C | - | 16.05 |
| prM/M | 568 | T | C | - | 3.50 | NS3 | 5340 | C | T | - | 15.85 |
| prM/M | 606 | C | T | - | 44.26 | NS3 | 5364 | T | C | - | 8.54 |
| prM/M | 651 | G | A | - | 29.63 | NS3 | 5367 | C | T | - | 8.43 |
| prM/M | 717 | A | G | - | 26.32 | NS3 | 5373 | C | T | - | 8.86 |
| prM/M | 792 | G | A | - | 49.35 | NS3 | 5382 | C | T | - | 9.21 |
| prM/M | 798 | T | C | - | 46.99 | NS3 | 5409 | T | A | - | 8.16 |
| prM/M | 834 | T | C | - | 40.91 | NS3 | 5508 | T | C | - | 9.09 |
| prM/M | 837 | T | C | - | 45.90 | NS3 | 5526 | G | A | - | 7.27 |
| prM/M | 855 | C | T | - | 6.35 | NS3 | 5532 | A | G | - | 8.93 |
| prM/M | 864 | C | T | - | 37.60 | NS3 | 5544 | G | A | - | 12.28 |
| prM/M | 873 | A | C | - | 40.83 | NS3 | 5550 | A | G | - | 12.73 |
| prM/M | 876 | G | A | - | 15.38 | NS3 | 5559 | A | T | - | 11.29 |
| prM/M | 880 | C | T | His148Tyr | 14.52 | NS3 | 5574 | C | T | - | 6.67 |
| prM/M | 882 | C | T | - | 12.59 | NS3 | 5583 | T | C | - | 9.84 |
| prM/M | 891 | G | A | - | 14.39 | NS3 | 5613 | T | C | - | 35.29 |
| prM/M | 893 | C | T | Ala152Val | 14.93 | NS3 | 5646 | T | G | - | 14.29 |
| prM/M | 894 | C | A | - | 28.57 | NS3 | 5649 | G | A | - | 13.89 |
| E | 954 | A | C | - | 25.00 | NS3 | 5688 | T | C | - | 9.26 |
| E | 960 | T | C | - | 14.93 | NS3 | 5694 | C | T | - | 7.41 |
| E | 969 | C | T | - | 13.53 | NS3 | 5697 | T | C | - | 17.54 |
| E | 972 | A | G | - | 13.33 | NS3 | 5730 | C | T | - | 17.54 |
| E | 1008 | T | C | - | 11.72 | NS3 | 5742 | A | G | - | 7.02 |
| E | 1023 | C | T | - | 39.13 | NS3 | 5766 | C | T | - | 10.64 |
| E | 1032 | G | A | - | 11.34 | NS3 | 5775 | G | A | - | 10.91 |
| E | 1040 | C | CA | Asn37fs | 3.45 | NS3 | 5793 | C | T | - | 9.23 |
| E | 1062 | T | C | - | 11.11 | NS3 | 5796 | T | C | - | 7.81 |
| E | 1068 | A | G | - | 11.94 | NS3 | 5802 | C | T | - | 37.74 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1089 | A | G | - | 11.11 | NS3 | 5805 | C | T | - | 6.78 |
| E | 1094 | C | T | Pro53Leu | 18.18 | NS3 | 5817 | T | C | - | 9.84 |
| E | 1102 | C | T | - | 47.37 | NS3 | 5823 | A | G | - | 7.69 |
| E | 1122 | A | G | - | 13.51 | NS3 | 5829 | T | C | - | 6.45 |
| E | 1207 | A | G | Ile91Val | 16.00 | NS3 | 5832 | C | T | - | 6.45 |
| E | 1214 | A | G | Lys93Arg | 17.86 | NS3 | 5835 | A | G | - | 40.98 |
| E | 1221 | C | T | - | 15.38 | NS3 | 5841 | G | A | - | 5.45 |
| E | 1287 | T | C | - | 9.38 | NS3 | 5859 | T | C | - | 33.33 |
| E | 1353 | C | T | - | 7.02 | NS3 | 5889 | A | G | - | 13.79 |
| E | 1381 | C | A | His149Asn | 10.26 | NS3 | 5958 | C | G | - | 10.53 |
| E | 1383 | C | T | - | 8.89 | NS3 | 6009 | T | C | - | 47.37 |
| E | 1389 | A | T | - | 8.89 | NS3 | 6033 | C | T | - | 40.28 |
| E | 1392 | T | A | - | 8.33 | NS3 | 6048 | C | T | - | 4.48 |
| E | 1396 | G | A | Asp154Asn | 18.60 | NS3 | 6063 | A | G | - | 5.26 |
| E | 1407 | G | A | - | 9.80 | NS3 | 6066 | A | G | - | 5.36 |
| E | 1413 | C | A | - | 27.45 | NS3 | 6069 | G | T | - | 46.55 |
| E | 1528 | C | T | - | 4.92 | NS3 | 6087 | A | G | - | 8.45 |
| E | 1536 | G | A | - | 3.94 | NS3 | 6093 | T | C | - | 6.56 |
| E | 1543 | G | A | Asp203Asn | 3.76 | NS3 | 6094 | T | C | - | 44.26 |
| E | 1545 | C | T | - | 41.09 | NS3 | 6096 | G | A | - | 6.45 |
| E | 1599 | A | G | - | 48.74 | NS3 | 6099 | A | G | - | 43.94 |
| E | 1626 | C | T | - | 33.33 | NS3 | 6108 | A | G | - | 10.45 |
| E | 1641 | G | A | - | 34.15 | NS3 | 6141 | G | A | - | 14.44 |
| E | 1722 | G | A | - | 4.60 | NS3 | 6157 | C | T | - | 32.18 |
| E | 1728 | C | T | - | 5.68 | NS3 | 6159 | G | A | - | 41.05 |
| E | 1737 | T | C | - | 8.14 | NS3 | 6167 | A | G | Lys549Arg | 21.50 |
| E | 1740 | A | G | - | 7.87 | NS3 | 6177 | T | C | - | 21.62 |
| E | 1765 | T | C | - | 11.39 | NS3 | 6186 | C | T | - | 15.65 |
| E | 1776 | G | A | - | 13.10 | NS3 | 6192 | C | T | - | 12.38 |
| E | 1858 | A | G | Ile308Val | 19.35 | NS3 | 6203 | A | G | Lys588Arg | 11.88 |
| E | 1860 | C | T | - | 20.69 | NS3 | 6222 | T | C | - | 38.89 |
| E | 1863 | G | A | - | 34.48 | NS3 | 6237 | A | C | - | 14.61 |
| E | 1920 | G | T | - | 13.51 | NS3 | 6238 | C | T | - | 12.77 |
| E | 1923 | C | T | - | 38.89 | NS3 | 6262 | A | G | Ile581Val | 10.26 |
| E | 1938 | G | A | - | 21.28 | NS3 | 6279 | G | A | - | 16.22 |
| E | 1944 | C | T | - | 47.92 | NS3 | 6288 | A | G | - | 16.67 |
| E | 1955 | C | T | Thr340Ile | 18.18 | NS3 | 6294 | A | G | - | 16.42 |
| E | 1956 | A | G | - | 18.18 | NS3 | 6300 | C | T | - | 33.33 |
| E | 1956 | A | T | - | 7.27 | NS3 | 6303 | A | G | - | 13.89 |
| E | 1959 | C | T | - | 43.64 | NS3 | 6307 | T | C | - | 13.51 |
| E | 1960 | T | C | - | 17.54 | NS3 | 6312 | T | C | - | 12.99 |
| E | 1972 | C | T | His346Tyr | 36.84 | NS3 | 6333 | A | G | - | 11.58 |
| E | 1974 | C | T | - | 18.03 | NS3 | 6339 | A | G | - | 10.48 |
| E | 1983 | A | T | - | 7.14 | NS3 | 6342 | A | C | - | 10.58 |
| E | 1995 | A | G | - | 19.40 | NS3 | 6354 | G | A | - | 10.83 |
| E | 1998 | T | C | - | 6.94 | NS3 | 6357 | A | G | - | 10.08 |
| E | 2007 | C | T | - | 5.06 | NS3 | 6366 | T | C | - | 12.40 |
| E | 2010 | A | C | - | 5.13 | NS4A | 6378 | T | C | - | 31.48 |
| E | 2064 | C | T | - | 8.33 | NS4A | 6379 | T | C | - | 30.84 |
| E | 2082 | A | G | - | 15.22 | NS4A | 6390 | C | T | - | 12.10 |
| E | 2100 | A | G | - | 9.26 | NS4A | 6423 | C | T | - | 9.68 |
| E | 2118 | G | A | - | 10.34 | NS4A | 6429 | T | C | - | 10.31 |
| E | 2124 | T | C | - | 10.71 | NS4A | 6435 | G | A | - | 7.07 |
| E | 2127 | C | A | - | 10.91 | NS4A | 6441 | A | G | - | 6.25 |
| E | 2142 | T | C | - | 10.34 | NS4A | 6444 | C | T | - | 6.38 |
| E | 2163 | A | G | - | 16.88 | NS4A | 6448 | C | T | - | 5.08 |
| E | 2175 | C | T | - | 17.07 | NS4A | 6462 | T | C | - | 30.17 |
| E | 2223 | T | C | - | 46.08 | NS4A | 6491 | A | G | Lys39Arg | 33.56 |
| E | 2241 | T | C | - | 16.54 | NS4A | 6496 | T | C | Tyr41His | 27.70 |
| E | 2250 | A | G | - | 17.65 | NS4A | 6500 | C | A | Thr42Asn | 26.62 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 2256 | C | T | - | 30.08 | NS4A | 6504 | T | C | - | 4.76 |
| E | 2276 | C | T | Ala447Val | 4.97 | NS4A | 6512 | A | G | Asn46Ser | 36.64 |
| E | 2277 | T | C | - | 10.56 | NS4A | 6519 | G | C | - | 4.35 |
| E | 2280 | T | C | - | 10.90 | NS4A | 6528 | T | C | - | 33.85 |
| E | 2283 | T | C | - | 11.95 | NS4A | 6538 | C | T | - | 4.14 |
| E | 2286 | G | A | - | 10.97 | NS4A | 6547 | C | T | - | 33.33 |
| E | 2289 | C | T | - | 32.32 | NS4A | 6556 | C | T | - | 37.59 |
| E | 2298 | T | C | - | 10.18 | NS4A | 6562 | G | A | Ala63Thr | 38.17 |
| E | 2307 | C | T | - | 44.00 | NS4A | 6582 | C | T | - | 3.07 |
| E | 2318 | T | C | Val461Ala | 23.56 | NS4A | 6588 | C | T | - | 3.55 |
| E | 2319 | C | A | - | 32.56 | NS4A | 6591 | A | G | - | 4.00 |
| E | 2319 | C | T | - | 8.14 | NS4A | 6615 | G | A | - | 8.50 |
| E | 2320 | A | G | Ile462Val | 7.18 | NS4A | 6645 | A | G | - | 11.38 |
| E | 2349 | T | C | - | 5.58 | NS4A | 6648 | T | C | - | 13.22 |
| E | 2367 | G | A | - | 6.57 | NS4A | 6651 | C | T | - | 11.02 |
| E | 2371 | C | T | - | 6.67 | NS4A | 6654 | T | C | - | 41.67 |
| E | 2376 | A | G | - | 28.27 | NS4A | 6666 | T | C | - | 19.15 |
| E | 2379 | G | A | - | 8.46 | NS4A | 6678 | A | G | - | 22.09 |
| E | 2395 | C | T | - | 9.24 | NS4A | 6681 | A | G | - | 30.86 |
| E | 2400 | C | T | - | 9.15 | NS4A | 6693 | A | G | - | 24.36 |
| E | 2401 | C | T | - | 9.49 | NS4A | 6727 | T | C | - | 11.25 |
| E | 2408 | C | T | Ala491Val | 9.66 | NS4A | 6732 | C | T | - | 31.33 |
| E | 2409 | T | C | - | 10.49 | NS4A | 6777 | G | A | - | 17.57 |
| NS1 | 2433 | C | T | - | 9.02 | NS4A | 6780 | C | T | - | 17.57 |
| NS1 | 2442 | C | T | - | 8.03 | NS4B | 6837 | T | G | - | 15.56 |
| NS1 | 2448 | A | G | - | 38.71 | NS4B | 6840 | C | T | - | 16.47 |
| NS1 | 2481 | C | T | - | 6.59 | NS4B | 6841 | C | T | - | 48.10 |
| NS1 | 2490 | T | C | - | 6.54 | NS4B | 6852 | C | G | - | 16.67 |
| NS1 | 2493 | C | T | - | 5.92 | NS4B | 6861 | C | T | - | 12.90 |
| NS1 | 2517 | T | C | - | 5.81 | NS4B | 6862 | C | T | Leu13Phe | 13.04 |
| NS1 | 2520 | G | A | - | 5.76 | NS4B | 6870 | T | G | Phe15Leu | 14.00 |
| NS1 | 2538 | T | C | - | 6.56 | NS4B | 6880 | A | G | Thr19Ala | 7.69 |
| NS1 | 2544 | A | G | - | 6.35 | NS4B | 6889 | G | C | Glu22Gln | 6.30 |
| NS1 | 2547 | A | G | - | 6.15 | NS4B | 6891 | A | G | - | 6.40 |
| NS1 | 2559 | C | T | - | 5.50 | NS4B | 6892 | T | C | Ser23Pro | 6.61 |
| NS1 | 2608 | T | C | - | 8.25 | NS4B | 6894 | T | C | - | 6.78 |
| NS1 | 2619 | G | A | - | 6.98 | NS4B | 6924 | T | C | - | 8.46 |
| NS1 | 2682 | C | T | - | 6.17 | NS4B | 6942 | G | C | - | 6.91 |
| NS1 | 2685 | T | C | - | 11.25 | NS4B | 6951 | C | A | - | 5.95 |
| NS1 | 2697 | C | T | - | 11.69 | NS4B | 6957 | T | C | - | 33.47 |
| NS1 | 2700 | T | C | - | 8.97 | NS4B | 6967 | G | A | Val48Ile | 2.28 |
| NS1 | 2731 | T | C | - | 9.43 | NS4B | 6979 | T | C | - | 2.26 |
| NS1 | 2778 | A | C | - | 7.14 | NS4B | 6982 | C | A | - | 2.47 |
| NS1 | 2791 | C | T | Leu124Phe | 6.17 | NS4B | 6990 | C | T | - | 2.56 |
| NS1 | 2835 | T | C | - | 10.53 | NS4B | 7008 | G | A | - | 29.74 |
| NS1 | 2850 | C | T | - | 17.02 | NS4B | 7014 | C | G | - | 4.79 |
| NS1 | 2878 | C | A | Leu153Met | 40.00 | NS4B | 7017 | C | T | - | 5.28 |
| NS1 | 2880 | G | A | - | 8.33 | NS4B | 7018 | C | T | - | 27.90 |
| NS1 | 2898 | T | C | - | 44.23 | NS4B | 7032 | T | C | - | 6.88 |
| NS1 | 2911 | A | T | Thr164Ser | 9.26 | NS4B | 7038 | A | G | - | 9.17 |
| NS1 | 2928 | A | G | - | 6.25 | NS4B | 7059 | T | C | - | 46.07 |
| NS1 | 2942 | A | G | Lys174Arg | 5.45 | NS4B | 7083 | G | A | - | 13.21 |
| NS1 | 2943 | A | G | - | 5.45 | NS4B | 7089 | C | T | - | 13.07 |
| NS1 | 2961 | C | T | - | 49.30 | NS4B | 7092 | C | T | - | 13.27 |
| NS1 | 2976 | A | G | - | 40.85 | NS4B | 7101 | C | T | - | 10.24 |
| NS1 | 2989 | G | A | Asp190Asn | 41.46 | NS4B | 7104 | C | T | - | 10.56 |
| NS1 | 3009 | C | T | - | 43.90 | NS4B | 7107 | T | C | - | 12.00 |
| NS1 | 3033 | T | C | - | 6.82 | NS4B | 7110 | C | T | - | 11.18 |
| NS1 | 3039 | C | T | - | 5.38 | NS4B | 7116 | A | G | - | 14.29 |
| NS1 | 3057 | G | A | Met212Ile | 6.76 | NS4B | 7122 | C | T | - | 16.92 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS1 | 3081 | T | A | - | 25.37 | NS4B | 7143 | C | T | - | 17.48 |
| NS1 | 3111 | C | T | - | 12.24 | NS4B | 7146 | C | T | - | 19.15 |
| NS1 | 3120 | C | T | - | 17.31 | NS4B | 7155 | T | C | - | 21.11 |
| NS1 | 3129 | A | G | - | 12.50 | NS4B | 7182 | C | T | - | 15.28 |
| NS1 | 3130 | T | C | - | 12.28 | NS4B | 7185 | T | C | - | 15.94 |
| NS1 | 3148 | A | G | Ile243Val | 15.63 | NS4B | 7186 | A | G | ile121Val | 26.09 |
| NS1 | 3156 | A | G | - | 18.75 | NS4B | 7200 | T | C | - | 15.38 |
| NS1 | 3165 | C | T | - | 18.75 | NS4B | 7206 | A | G | - | 12.24 |
| NS1 | 3168 | G | A | - | 15.19 | NS4B | 7224 | T | G | - | 7.69 |
| NS1 | 3174 | G | A | - | 16.28 | NS4B | 7248 | C | T | - | 26.00 |
| NS1 | 3186 | C | T | - | 9.80 | NS4B | 7314 | A | G | - | 47.83 |
| NS1 | 3201 | C | T | - | 7.69 | NS4B | 7348 | A | G | Ile175Val | 4.42 |
| NS1 | 3204 | T | C | - | 7.76 | NS4B | 7356 | C | T | - | 49.56 |
| NS1 | 3213 | A | G | - | 10.19 | NS4B | 7371 | A | G | - | 4.13 |
| NS1 | 3222 | T | C | - | 10.48 | NS4B | 7410 | C | T | - | 24.07 |
| NS1 | 3258 | C | T | - | 11.72 | NS4B | 7449 | T | C | - | 42.86 |
| NS1 | 3261 | C | T | - | 11.63 | NS5 | 7599 | A | G | - | 39.13 |
| NS1 | 3270 | T | C | - | 10.90 | NS5 | 7686 | C | T | - | 17.86 |
| NS1 | 3289 | G | A | Asp290Asn | 6.54 | NS5 | 7794 | G | A | - | 37.04 |
| NS1 | 3313 | T | C | - | 8.70 | NS5 | 7848 | A | C | - | 9.68 |
| NS1 | 3324 | C | T | - | 7.30 | NS5 | 7890 | A | G | - | 11.49 |
| NS1 | 3327 | T | C | - | 8.57 | NS5 | 7899 | C | T | - | 11.24 |
| NS1 | 3330 | C | T | - | 43.07 | NS5 | 7908 | C | T | - | 8.42 |
| NS1 | 3339 | G | A | - | 5.97 | NS5 | 7911 | C | T | - | 46.24 |
| NS1 | 3342 | C | T | - | 36.72 | NS5 | 7914 | C | A | - | 7.92 |
| NS1 | 3357 | C | T | - | 4.92 | NS5 | 7947 | G | T | - | 2.61 |
| NS1 | 3366 | C | T | - | 8.15 | NS5 | 7999 | T | C | - | 5.64 |
| NS1 | 3373 | C | T | - | 6.15 | NS5 | 8035 | A | G | Ile156Val | 4.08 |
| NS1 | 3381 | T | G | - | 6.25 | NS5 | 8037 | A | T | - | 4.17 |
| NS1 | 3408 | C | T | - | 5.66 | NS5 | 8049 | A | G | - | 43.35 |
| NS2A | 3489 | G | A | - | 26.53 | NS5 | 8064 | C | A | - | 2.80 |
| NS2A | 3595 | T | C | Phe40Leu | 19.75 | NS5 | 8070 | A | G | - | 3.35 |
| NS2A | 3604 | T | C | - | 22.47 | NS5 | 8083 | C | T | - | 37.50 |
| NS2A | 3612 | T | A | - | 11.54 | NS5 | 8088 | C | T | - | 3.57 |
| NS2A | 3663 | T | C | - | 46.92 | NS5 | 8091 | T | C | - | 3.57 |
| NS2A | 3750 | A | G | - | 7.41 | NS5 | 8115 | C | T | - | 3.92 |
| NS2A | 3753 | C | T | - | 41.12 | NS5 | 8121 | T | C | - | 33.09 |
| NS2A | 3768 | C | T | - | 44.74 | NS5 | 8139 | C | T | - | 5.10 |
| NS2A | 3787 | G | A | Ala104Thr | 3.41 | NS5 | 8184 | A | G | - | 36.90 |
| NS2A | 3807 | C | T | - | 1.72 | NS5 | 8208 | C | T | - | 4.12 |
| NS2A | 3834 | C | T | - | 4.18 | NS5 | 8211 | C | T | - | 29.67 |
| NS2A | 3837 | T | A | - | 3.64 | NS5 | 8214 | G | A | - | 10.00 |
| NS2A | 3840 | A | T | - | 3.59 | NS5 | 8220 | A | G | - | 6.73 |
| NS2A | 3844 | C | T | - | 2.67 | NS5 | 8226 | C | T | - | 5.41 |
| NS2A | 3858 | A | G | - | 11.04 | NS5 | 8235 | T | C | - | 11.11 |
| NS2A | 3874 | G | A | Val133Ile | 9.00 | NS5 | 8241 | T | C | - | 42.27 |
| NS2A | 3876 | C | T | - | 9.62 | NS5 | 8242 | A | T | Thr225Ser | 8.08 |
| NS2A | 3900 | A | G | - | 10.00 | NS5 | 8284 | T | C | - | 37.08 |
| NS2A | 3903 | A | G | - | 10.36 | NS5 | 8289 | T | C | - | 16.85 |
| NS2A | 3906 | C | T | - | 9.60 | NS5 | 8298 | C | T | - | 17.14 |
| NS2A | 3912 | G | A | - | 9.92 | NS5 | 8306 | A | G | Lys246Arg | 25.37 |
| NS2A | 3928 | G | C | Ala151Pro | 1.84 | NS5 | 8310 | T | C | - | 26.79 |
| NS2A | 3935 | C | T | Ser153Leu | 8.45 | NS5 | 8316 | A | G | - | 18.60 |
| NS2A | 3936 | G | A | - | 8.41 | NS5 | 8322 | C | A | - | 22.86 |
| NS2A | 3942 | C | T | - | 9.51 | NS5 | 8343 | A | C | - | 26.32 |
| NS2A | 3958 | T | C | - | 7.94 | NS5 | 8349 | T | C | - | 36.84 |
| NS2A | 3960 | G | A | - | 7.76 | NS5 | 8364 | T | C | - | 43.75 |
| NS2A | 3966 | C | T | - | 7.89 | NS5 | 8481 | T | C | - | 16.13 |
| NS2A | 3975 | G | A | - | 7.77 | NS5 | 8496 | T | C | - | 14.29 |
| NS2A | 3981 | T | C | - | 7.45 | NS5 | 8508 | A | G | - | 16.98 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2A | 3991 | T | C | - | 7.88 | NS5 | 8526 | T | C | - | 20.31 |
| NS2A | 3998 | C | T | Ala174Val | 9.61 | NS5 | 8535 | C | T | - | 19.70 |
| NS2A | 4009 | T | A | Ser178Thr | 9.82 | NS5 | 8541 | G | A | - | 17.39 |
| NS2A | 4021 | C | T | - | 8.22 | NS5 | 8572 | G | A | Val335Ile | 23.64 |
| NS2A | 4047 | T | C | - | 6.41 | NS5 | 8577 | T | C | - | 21.67 |
| NS2A | 4057 | C | T | - | 6.73 | NS5 | 8604 | C | T | - | 37.50 |
| NS2A | 4071 | A | C | - | 6.46 | NS5 | 8610 | T | C | - | 25.64 |
| NS2A | 4074 | A | G | - | 6.69 | NS5 | 8634 | C | T | - | 40.00 |
| NS2A | 4083 | C | T | - | 46.32 | NS5 | 8658 | T | C | - | 40.00 |
| NS2A | 4098 | T | C | - | 8.43 | NS5 | 8745 | G | A | - | 21.43 |
| NS2A | 4107 | T | C | - | 8.37 | NS5 | 8751 | C | T | - | 17.65 |
| NS2A | 4110 | T | C | - | 30.04 | NS5 | 8754 | T | C | - | 17.65 |
| NS2A | 4113 | G | A | - | 8.30 | NS5 | 8775 | G | A | - | 26.32 |
| NS2A | 4121 | G | A | Ser215Asn | 9.91 | NS5 | 8794 | T | C | - | 21.43 |
| NS2B | 4134 | C | T | - | 8.22 | NS5 | 8796 | G | A | - | 23.08 |
| NS2B | 4140 | G | A | - | 10.41 | NS5 | 8799 | G | T | - | 20.69 |
| NS2B | 4149 | A | G | - | 11.74 | NS5 | 8823 | A | G | - | 7.32 |
| NS2B | 4164 | C | T | - | 37.50 | NS5 | 8859 | G | T | Arg430Ser | 20.93 |
| NS2B | 4197 | A | G | - | 7.88 | NS5 | 8967 | C | T | - | 43.75 |
| NS2B | 4212 | T | C | - | 6.80 | NS5 | 8979 | T | C | - | 34.88 |
| NS2B | 4221 | T | A | - | 5.22 | NS5 | 9012 | A | G | - | 24.53 |
| NS2B | 4225 | T | C | - | 5.19 | NS5 | 9018 | C | T | - | 7.41 |
| NS2B | 4242 | C | T | - | 7.37 | NS5 | 9021 | A | G | - | 8.00 |
| NS2B | 4248 | C | T | - | 5.03 | NS5 | 9042 | T | C | - | 31.71 |
| NS2B | 4251 | A | G | - | 7.07 | NS5 | 9043 | C | T | - | 9.76 |
| NS2B | 4270 | C | A | - | 6.00 | NS5 | 9063 | C | T | - | 16.28 |
| NS2B | 4305 | T | C | - | 9.38 | NS5 | 9071 | G | A | Gly501Glu | 16.67 |
| NS2B | 4310 | A | G | Lys60Arg | 11.00 | NS5 | 9078 | C | T | - | 22.22 |
| NS2B | 4323 | G | A | - | 13.25 | NS5 | 9309 | G | A | - | 25.00 |
| NS2B | 4335 | A | G | - | 16.00 | NS5 | 9399 | C | T | - | 18.75 |
| NS2B | 4341 | C | T | - | 25.00 | NS5 | 9576 | T | C | - | 38.94 |
| NS2B | 4350 | C | T | - | 45.61 | NS5 | 9708 | T | C | - | 4.69 |
| NS2B | 4368 | A | G | - | 26.83 | NS5 | 9810 | T | C | - | 3.86 |
| NS2B | 4386 | G | A | - | 27.59 | NS5 | 9829 | T | C | - | 4.07 |
| NS2B | 4401 | G | A | - | 27.59 | NS5 | 9834 | G | A | - | 4.57 |
| NS2B | 4414 | C | T | - | 25.00 | NS5 | 9837 | G | A | - | 4.19 |
| NS2B | 4434 | G | A | - | 16.13 | NS5 | 9857 | C | G | Thr763Ser | 4.85 |
| NS2B | 4438 | T | C | - | 38.24 | NS5 | 9870 | C | T | - | 5.48 |
| NS2B | 4441 | T | C | - | 14.71 | NS5 | 9888 | A | G | - | 31.22 |
| NS2B | 4449 | C | A | - | 10.87 | NS5 | 9889 | T | C | - | 7.53 |
| NS2B | 4456 | G | C | Val109Leu | 9.43 | NS5 | 9900 | T | C | - | 8.43 |
| NS2B | 4458 | C | T | - | 9.62 | NS5 | 9903 | C | T | - | 8.54 |
| NS2B | 4464 | A | C | - | 11.11 | NS5 | 9921 | G | A | - | 9.15 |
| NS2B | 4470 | G | A | - | 11.54 | NS5 | 9927 | T | C | - | 7.14 |
| NS2B | 4479 | T | C | - | 11.86 | NS5 | 10075 | A | G | Ile836Val | 45.69 |
| NS2B | 4487 | C | T | Ala119Val | 11.48 | NS5 | 10083 | C | T | - | 8.63 |
| NS2B | 4509 | G | T | - | 39.06 | NS5 | 10089 | G | A | - | 28.81 |
| NS2B | 4512 | G | A | - | 21.54 | NS5 | 10252 | G | A | Glu895Lys | 28.57 |
| NS3 | 4530 | A | G | - | 17.91 | 3UTR | 10369 | A | T | - | 3.97 |
| NS3 | 4539 | C | T | - | 32.56 | 3UTR | 10387 | C | T | - | 3.80 |
| NS3 | 4542 | C | T | - | 29.79 | 3UTR | 10389 | C | CA | - | 16.56 |
| NS3 | 4557 | A | C | - | 28.07 | 3UTR | 10389 | C | CAA | - | 5.30 |
| NS3 | 4562 | G | A | Gly14Glu | 18.52 | 3UTR | 10389 | C | T | - | 4.00 |
| NS3 | 4566 | A | G | - | 28.30 | 3UTR | 10400 | G | A | - | 3.90 |
| NS3 | 4573 | C | T | - | 27.78 | 3UTR | 10407 | T | C | - | 29.53 |
| NS3 | 4596 | C | T | - | 47.62 | 3UTR | 10411 | C | T | - | 4.00 |
| NS3 | 4602 | A | G | - | 13.16 | 3UTR | 10413 | G | A | - | 4.23 |
| NS3 | 4604 | G | A | Arg28Lys | 13.51 | 3UTR | 10435 | A | G | - | 6.14 |
| NS3 | 4620 | T | C | - | 12.50 | 3UTR | 10437 | G | A | - | 6.10 |
| NS3 | 4623 | T | C | - | 13.51 | 3UTR | 10450 | C | T | - | 8.22 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS3 | 4629 | T | C | - | 14.29 | 3UTR | 10452 | C | T | - | 8.33 |

| **201** | | | | | |
|---|---|---|---|---|---|
| SD | | | | | |
| Secondary | | | | | |
| 3.23E+00 | | | | | |
| 4 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 127 | A | T | Thr11Ser | 16.67 |
| C | 219 | C | T | - | 5.26 |
| C | 231 | A | G | - | 13.85 |
| prM/M | 606 | T | C | - | 14.86 |
| prM/M | 717 | G | A | - | 26.32 |
| prM/M | 885 | C | T | - | 11.58 |
| E | 1094 | C | T | Pro53Leu | 48.60 |
| E | 1214 | A | G | Lys93Arg | 10.81 |
| E | 1287 | T | C | - | 11.59 |
| E | 1834 | G | T | Ala300Ser | 32.00 |
| E | 1956 | A | T | - | 13.33 |
| E | 2082 | A | G | - | 47.37 |
| E | 2223 | C | T | - | 7.87 |
| E | 2256 | T | C | - | 23.88 |
| E | 2276 | C | T | Ala447Val | 16.90 |
| E | 2318 | C | T | Ala461Val | 25.69 |
| NS1 | 2448 | G | A | - | 9.43 |
| NS1 | 2685 | T | C | - | 21.88 |
| NS1 | 2731 | T | C | - | 11.11 |
| NS1 | 2791 | C | T | Leu124Phe | 9.68 |
| NS1 | 3009 | C | T | - | 6.67 |
| NS1 | 3148 | G | A | val243Ile | 38.64 |
| NS1 | 3162 | T | A | Phe247Leu | 7.69 |
| NS1 | 3342 | T | C | - | 32.53 |
| NS2A | 3489 | G | A | - | 35.19 |
| NS2A | 3595 | T | C | Phe40Leu | 19.12 |
| NS2A | 3612 | T | A | - | 15.38 |
| NS2A | 3663 | T | C | - | 5.94 |
| NS2A | 3750 | A | G | - | 15.09 |
| NS2A | 3801 | C | T | - | 11.31 |
| NS2A | 3858 | A | G | - | 7.49 |
| NS2A | 3910 | T | C | - | 6.56 |
| NS2A | 4110 | C | T | - | 21.83 |
| NS2B | 4249 | G | A | Val40Ile | 4.62 |
| NS2B | 4257 | C | T | - | 3.80 |
| NS2B | 4341 | C | T | - | 8.89 |
| NS3 | 4562 | G | A | Gly14Glu | 33.33 |
| NS3 | 4974 | C | T | - | 28.72 |
| NS3 | 5697 | T | C | - | 25.00 |
| NS3 | 5730 | C | T | - | 30.61 |
| NS3 | 5775 | G | A | - | 9.68 |
| NS3 | 5793 | C | T | - | 11.11 |
| NS3 | 5823 | A | G | - | 13.95 |
| NS3 | 6157 | T | C | - | 14.00 |
| NS3 | 6262 | G | A | Val581Ile | 34.29 |
| NS3 | 6333 | A | G | - | 6.00 |
| NS4A | 6378 | T | C | - | 1.19 |
| NS4A | 6471 | T | C | - | 12.16 |
| NS4A | 6585 | A | G | - | 7.46 |
| NS4A | 6727 | T | C | - | 19.61 |
| NS4B | 6871 | G | A | Gly16Arg | 6.17 |
| NS4B | 6951 | C | A | - | 9.57 |
| NS4B | 7038 | A | G | - | 7.18 |
| NS4B | 7449 | C | T | - | 27.54 |
| NS5 | 7599 | A | G | - | 38.46 |
| NS5 | 7999 | T | C | - | 10.79 |
| NS5 | 8139 | C | T | - | 19.30 |
| NS5 | 8211 | T | C | - | 41.27 |
| NS5 | 8214 | G | A | - | 19.05 |
| NS5 | 8235 | T | C | - | 14.29 |
| NS5 | 8604 | T | C | - | 34.21 |
| NS5 | 9042 | T | C | - | 34.38 |
| NS5 | 9749 | C | A | Pro727Gln | 3.19 |
| NS5 | 9810 | T | C | - | 5.65 |
| NS5 | 10083 | C | T | - | 23.81 |
| NS5 | 10089 | A | G | - | 29.03 |
| 3UTR | 10387 | C | CAT | - | 5.56 |
| 3UTR | 10389 | CA | C | - | 29.63 |
| 3UTR | 10389 | C | CA | - | 12.96 |
| 3UTR | 10408 | C | T | - | 14.55 |
| 3UTR | 10444 | T | TA | - | 5.71 |

| **202** | | | | | |
|---|---|---|---|---|---|
| SD | | | | | |
| Primary | | | | | |
| 3.45E+04 | | | | | |
| ND | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 70 | G | GT | - | 2.15 |
| C | 110 | G | GA | Lys6fs | 0.98 |
| C | 135 | C | T | - | 1.04 |
| C | 187 | A | C | Lys31Gln | 0.66 |
| C | 341 | G | T | Arg82Ile | 1.77 |
| C | 431 | C | T | Ala112Val | 0.88 |
| prM/M | 445 | T | C | - | 0.66 |
| prM/M | 508 | C | T | - | 0.55 |
| prM/M | 522 | G | A | - | 1.04 |
| prM/M | 523 | G | A | Asp29Asn | 1.10 |
| NS3 | 4635 | C | T | - | 1.08 |
| NS3 | 4641 | T | C | - | 1.18 |
| NS3 | 4806 | T | C | - | 2.14 |
| NS3 | 4837 | C | T | Pro106Ser | 1.89 |
| NS3 | 4905 | G | A | - | 0.81 |
| NS3 | 5026 | G | GA | Ser171fs | 7.00 |
| NS3 | 5182 | T | C | - | 2.02 |
| NS3 | 5235 | C | T | - | 2.49 |
| NS3 | 5277 | C | T | - | 0.85 |
| NS3 | 5304 | T | G | Cys261Trp | 0.88 |
| NS3 | 5433 | A | T | - | 1.43 |
| NS3 | 5436 | G | A | - | 2.07 |
| NS3 | 5451 | C | A | - | 1.27 |
| NS3 | 5460 | T | C | - | 1.19 |
| NS3 | 5531 | G | T | Arg337Ile | 0.63 |
| NS3 | 5610 | A | G | - | 0.90 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| prM/M | 525 | T | C | - | 1.06 | NS3 | 5717 | C | T | Ala399Val | 1.83 |
| prM/M | 531 | T | C | - | 1.31 | NS3 | 5766 | C | T | - | 0.99 |
| prM/M | 567 | G | A | - | 0.58 | NS3 | 5781 | G | A | - | 0.79 |
| prM/M | 651 | A | G | - | 2.04 | NS3 | 5841 | G | A | - | 0.75 |
| prM/M | 717 | G | A | - | 2.13 | NS3 | 5887 | C | T | Gln456* | 0.90 |
| prM/M | 792 | G | A | - | 0.66 | NS3 | 5889 | G | A | - | 0.80 |
| prM/M | 808 | G | A | Glu124Lys | 0.55 | NS3 | 5915 | C | CA | Asn467fs | 5.98 |
| prM/M | 837 | T | C | - | 1.05 | NS3 | 6048 | C | T | - | 0.59 |
| prM/M | 852 | G | A | - | 0.94 | NS3 | 6096 | G | A | - | 0.59 |
| prM/M | 856 | C | T | - | 0.90 | NS3 | 6141 | G | A | - | 1.16 |
| prM/M | 893 | C | T | Ala152Val | 0.53 | NS3 | 6157 | T | C | - | 2.54 |
| prM/M | 921 | T | C | - | 1.92 | NS3 | 6159 | G | A | - | 1.49 |
| E | 1001 | A | C | Asp22Ala | 0.52 | NS3 | 6167 | A | G | Lys549Arg | 1.18 |
| E | 1005 | C | A | - | 0.82 | NS3 | 6192 | C | T | - | 0.71 |
| E | 1023 | T | C | - | 1.22 | NS3 | 6216 | C | T | - | 2.43 |
| E | 1094 | C | T | Pro53Leu | 0.76 | NS3 | 6285 | G | A | - | 2.17 |
| E | 1293 | T | C | - | 1.09 | NS3 | 6285 | G | GA | Lys590fs | 1.86 |
| E | 1413 | A | C | - | 1.41 | NS4A | 6379 | T | C | - | 1.13 |
| E | 1545 | C | T | - | 1.47 | NS4A | 6504 | C | T | - | 2.01 |
| E | 1599 | A | G | - | 0.86 | NS4A | 6512 | G | A | Ser46Asn | 1.71 |
| E | 1626 | T | C | - | 1.49 | NS4A | 6519 | C | G | - | 2.00 |
| E | 1695 | A | G | - | 0.96 | NS4A | 6625 | G | A | Gly84Arg | 0.86 |
| E | 1944 | T | C | - | 0.91 | NS4A | 6633 | T | C | - | 1.16 |
| E | 1959 | T | C | - | 1.00 | NS4A | 6654 | T | C | - | 1.24 |
| E | 1972 | C | T | His346Tyr | 0.91 | NS4A | 6681 | A | G | - | 0.87 |
| E | 2064 | T | C | - | 1.18 | NS4B | 6840 | C | T | - | 1.05 |
| E | 2210 | T | A | Leu425* | 0.96 | NS4B | 6844 | G | GA | Thr9fs | 0.88 |
| E | 2223 | T | C | - | 0.92 | NS4B | 6844 | G | A | Glu7Lys | 0.77 |
| E | 2247 | T | C | - | 1.03 | NS4B | 6852 | C | G | - | 0.95 |
| E | 2256 | C | T | - | 0.59 | NS4B | 6861 | C | T | - | 0.86 |
| E | 2265 | T | C | - | 2.51 | NS4B | 6894 | T | C | - | 1.04 |
| E | 2271 | G | A | - | 2.77 | NS4B | 6922 | C | T | Arg33Cys | 1.15 |
| E | 2376 | A | G | - | 1.45 | NS4B | 6942 | G | C | - | 0.65 |
| NS1 | 2511 | G | A | - | 0.81 | NS4B | 6957 | T | C | - | 1.86 |
| NS1 | 2697 | C | T | - | 0.66 | NS4B | 6967 | G | A | Val48Ile | 0.64 |
| NS1 | 2776 | G | A | Gly119Ser | 0.74 | NS4B | 6982 | C | T | Arg53* | 1.00 |
| NS1 | 2808 | T | C | - | 1.04 | NS4B | 6982 | C | A | - | 0.58 |
| NS1 | 2898 | T | C | - | 0.64 | NS4B | 6990 | C | T | - | 0.73 |
| NS1 | 2952 | G | A | - | 1.21 | NS4B | 7008 | A | G | - | 1.45 |
| NS1 | 2989 | G | A | Asp190Asn | 1.33 | NS4B | 7014 | C | G | - | 0.74 |
| NS1 | 3009 | C | T | - | 1.20 | NS4B | 7017 | C | T | - | 0.78 |
| NS1 | 3060 | G | A | - | 0.78 | NS4B | 7018 | C | T | - | 1.54 |
| NS1 | 3075 | T | C | - | 1.52 | NS4B | 7032 | T | C | - | 0.81 |
| NS1 | 3081 | T | A | - | 1.09 | NS4B | 7041 | T | C | - | 1.40 |
| NS1 | 3102 | G | A | - | 1.24 | NS4B | 7062 | G | A | - | 9.83 |
| NS1 | 3111 | C | T | - | 0.99 | NS4B | 7083 | G | A | - | 0.63 |
| NS1 | 3114 | A | C | - | 2.30 | NS4B | 7089 | C | T | - | 0.57 |
| NS1 | 3120 | C | T | - | 1.26 | NS4B | 7092 | C | T | - | 0.62 |
| NS1 | 3129 | A | G | - | 1.47 | NS4B | 7122 | C | T | - | 0.59 |
| NS1 | 3130 | T | C | - | 1.27 | NS4B | 7137 | T | C | - | 0.97 |
| NS1 | 3152 | C | CA | Asn246fs | 3.76 | NS4B | 7152 | A | T | - | 0.94 |
| NS1 | 3165 | C | T | - | 1.39 | NS4B | 7186 | A | G | ile121Val | 1.42 |
| NS1 | 3168 | G | A | - | 1.23 | NS4B | 7383 | T | C | - | 1.01 |
| NS1 | 3186 | C | T | - | 0.74 | NS4B | 7428 | C | T | - | 1.13 |
| NS1 | 3234 | T | C | - | 0.92 | NS4B | 7435 | C | G | Leu204Val | 0.78 |
| NS1 | 3330 | C | T | - | 0.55 | NS5 | 7794 | G | A | - | 1.49 |
| NS1 | 3402 | T | C | - | 0.65 | NS5 | 7848 | A | C | - | 1.47 |
| NS1 | 3414 | T | C | - | 1.82 | NS5 | 7849 | T | C | - | 2.92 |
| NS2A | 3546 | T | C | - | 2.02 | NS5 | 7899 | C | T | - | 0.89 |
| NS2A | 3552 | C | T | - | 2.05 | NS5 | 7908 | C | T | - | 0.98 |

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|---|---|---|---|---|---|
| NS2A | 3600 | G | T | - | 1.69 |
| NS2A | 3604 | T | C | - | 1.16 |
| NS2A | 3624 | T | C | - | 0.83 |
| NS2A | 3629 | G | A | Arg51Lys | 0.71 |
| NS2A | 3663 | T | C | - | 1.59 |
| NS2A | 3753 | C | T | - | 0.54 |
| NS2A | 3864 | G | A | - | 0.60 |
| NS2A | 3875 | C | T | Ala133Val | 0.78 |
| NS2A | 3912 | G | A | - | 0.51 |
| NS2A | 3935 | C | T | Ser153Leu | 0.67 |
| NS2A | 3936 | G | A | - | 0.61 |
| NS2A | 3942 | C | T | - | 0.63 |
| NS2A | 3966 | C | T | - | 0.54 |
| NS2A | 3975 | G | A | - | 0.56 |
| NS2A | 3998 | C | T | Ala174Val | 0.68 |
| NS2A | 4011 | T | C | - | 1.14 |
| NS2A | 4034 | A | G | Gln186Arg | 1.09 |
| NS2A | 4047 | T | C | - | 0.64 |
| NS2A | 4063 | C | T | - | 3.59 |
| NS2A | 4071 | A | C | - | 0.69 |
| NS2A | 4074 | A | G | - | 0.72 |
| NS2A | 4083 | C | T | - | 1.50 |
| NS2A | 4098 | T | C | - | 0.59 |
| NS2B | 4164 | C | T | - | 0.71 |
| NS2B | 4197 | A | G | - | 0.83 |
| NS2B | 4206 | T | C | - | 1.44 |
| NS2B | 4212 | T | C | - | 0.92 |
| NS2B | 4278 | C | T | - | 0.84 |
| NS2B | 4329 | A | G | - | 1.12 |
| NS2B | 4344 | C | T | - | 0.98 |
| NS3 | 4539 | C | T | - | 0.94 |
| NS3 | 4573 | C | T | - | 0.83 |
| NS3 | 4602 | A | G | - | 1.18 |
| NS3 | 4604 | G | A | Arg28Lys | 1.01 |
| NS3 | 4620 | T | C | - | 1.16 |
| NS5 | 7911 | C | T | - | 1.56 |
| NS5 | 7914 | C | A | - | 0.95 |
| NS5 | 7959 | C | T | - | 1.15 |
| NS5 | 7971 | C | T | - | 0.51 |
| NS5 | 8074 | G | C | Glu169Gln | 0.93 |
| NS5 | 8204 | G | T | Arg212Leu | 0.94 |
| NS5 | 8242 | A | T | Thr225Ser | 0.72 |
| NS5 | 8256 | G | T | - | 1.23 |
| NS5 | 8284 | T | C | - | 1.05 |
| NS5 | 8289 | T | C | - | 1.08 |
| NS5 | 8306 | A | G | Lys246Arg | 0.98 |
| NS5 | 8310 | T | C | - | 1.02 |
| NS5 | 8891 | T | TA | His442fs | 1.01 |
| NS5 | 8919 | TG | T | Val451fs | 0.51 |
| NS5 | 8926 | A | AC | Asn453fs | 2.23 |
| NS5 | 9018 | T | C | - | 1.06 |
| NS5 | 9079 | C | T | - | 0.71 |
| NS5 | 9209 | T | TA | Asn549fs | 0.74 |
| NS5 | 9705 | T | TC | Phe713fs | 0.78 |
| NS5 | 9714 | G | C | Glu715Asp | 0.50 |
| NS5 | 9726 | AG | A | Asp720fs | 0.76 |
| NS5 | 9825 | T | C | - | 0.80 |
| NS5 | 9888 | A | G | - | 2.08 |
| NS5 | 9902 | C | G | Ala778Gly | 2.12 |
| NS5 | 10060 | A | G | Lys831Glu | 2.82 |
| NS5 | 10075 | G | A | Val836Ile | 1.63 |
| NS5 | 10083 | C | T | - | 1.14 |
| NS5 | 10155 | T | C | - | 1.72 |
| NS5 | 10202 | G | A | Gly878Glu | 1.66 |
| 3UTR | 10283 | C | T | - | 2.48 |
| 3UTR | 10443 | T | TA | - | 1.56 |
| 3UTR | 10450 | C | T | - | 0.67 |
| 3UTR | 10452 | C | T | - | 0.79 |
| 3UTR | 10463 | G | A | - | 4.47 |
| 3UTR | 10611 | C | CA | - | 2.57 |

| **203** | | | | | |
|---|---|---|---|---|---|
| SD | | | | | |
| Primary | | | | | |
| 4.76E+03 | | | | | |
| 5 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 70 | G | GT | - | 2.00 |
| C | 384 | C | T | - | 1.27 |
| prM/M | 445 | T | C | - | 2.24 |
| prM/M | 450 | C | T | - | 2.15 |
| prM/M | 508 | C | T | - | 1.12 |
| prM/M | 567 | G | A | - | 4.13 |
| prM/M | 651 | G | A | - | 2.69 |
| prM/M | 792 | G | A | - | 2.88 |
| prM/M | 798 | T | C | - | 2.73 |
| prM/M | 837 | T | C | - | 1.30 |
| prM/M | 852 | G | A | - | 1.57 |
| prM/M | 856 | C | T | - | 1.55 |
| prM/M | 864 | C | T | - | 1.97 |
| prM/M | 873 | A | C | - | 1.94 |
| prM/M | 894 | C | A | - | 2.26 |
| E | 954 | A | C | - | 2.52 |
| E | 1005 | C | A | - | 1.82 |
| NS2B | 4344 | C | T | - | 3.16 |
| NS2B | 4482 | G | A | - | 2.59 |
| NS2B | 4509 | G | T | - | 2.14 |
| NS3 | 4596 | C | T | - | 8.13 |
| NS3 | 4905 | G | A | - | 1.53 |
| NS3 | 4959 | T | C | - | 3.01 |
| NS3 | 5004 | C | T | - | 3.23 |
| NS3 | 5190 | C | T | - | 2.83 |
| NS3 | 5304 | T | C | - | 3.05 |
| NS3 | 5451 | C | A | - | 3.42 |
| NS3 | 5460 | T | C | - | 3.45 |
| NS3 | 5531 | G | T | Arg337Ile | 2.68 |
| NS3 | 5802 | C | T | - | 2.55 |
| NS3 | 5915 | C | CA | Asn467fs | 4.92 |
| NS3 | 6009 | T | C | - | 6.36 |
| NS3 | 6069 | G | T | - | 3.04 |
| NS3 | 6094 | T | C | - | 2.55 |
| NS3 | 6220 | A | G | Ile567Val | 7.42 |
| NS3 | 6222 | T | C | - | 3.52 |
| NS3 | 6285 | G | GA | Lys590fs | 2.06 |
| NS3 | 6300 | C | T | - | 2.24 |
| NS4A | 6378 | T | C | - | 2.24 |
| NS4A | 6379 | T | C | - | 2.23 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1094 | C | T | Pro53Leu | 2.08 | NS4A | 6462 | T | C | - | 3.75 |
| E | 1102 | C | T | - | 2.20 | NS4A | 6471 | T | C | - | 2.18 |
| E | 1293 | T | C | - | 3.72 | NS4A | 6491 | A | G | Lys39Arg | 2.92 |
| E | 1413 | A | C | - | 3.21 | NS4A | 6496 | T | C | Tyr41His | 3.24 |
| E | 1473 | C | T | - | 3.38 | NS4A | 6500 | C | A | Thr42Asn | 2.70 |
| E | 1590 | G | A | - | 4.68 | NS4A | 6528 | T | C | - | 3.29 |
| E | 1641 | G | A | - | 1.54 | NS4A | 6547 | C | T | - | 3.71 |
| E | 1995 | A | G | - | 2.56 | NS4A | 6556 | C | T | - | 3.86 |
| E | 2007 | C | T | - | 1.85 | NS4A | 6562 | G | A | Ala63Thr | 3.45 |
| E | 2023 | A | G | Ser363Gly | 2.08 | NS4A | 6585 | A | G | - | 3.68 |
| E | 2029 | A | G | Ile365Val | 5.75 | NS4A | 6633 | T | C | - | 6.23 |
| E | 2049 | C | T | - | 4.58 | NS4A | 6654 | T | C | - | 6.08 |
| E | 2064 | T | C | - | 3.87 | NS4A | 6681 | A | G | - | 4.27 |
| E | 2067 | C | T | - | 3.25 | NS4A | 6711 | G | GT | Leu115fs | 1.82 |
| E | 2073 | C | T | - | 4.00 | NS4B | 6844 | G | GA | Thr9fs | 1.08 |
| E | 2223 | T | C | - | 3.31 | NS4B | 6852 | C | CA | Lys11fs | 3.37 |
| E | 2247 | T | C | - | 3.28 | NS4B | 6864 | C | T | - | 2.52 |
| E | 2256 | C | T | - | 2.13 | NS4B | 6871 | G | A | Gly16Arg | 2.42 |
| E | 2289 | C | T | - | 3.50 | NS4B | 6948 | T | C | - | 3.58 |
| E | 2307 | C | T | - | 4.20 | NS4B | 6951 | C | A | - | 1.49 |
| E | 2319 | C | A | - | 3.82 | NS4B | 6982 | C | T | Arg53* | 1.99 |
| E | 2328 | A | T | - | 1.62 | NS4B | 7008 | A | G | - | 5.81 |
| NS1 | 2434 | A | G | Ile5Val | 4.32 | NS4B | 7041 | T | C | - | 5.42 |
| NS1 | 2448 | A | G | - | 3.95 | NS4B | 7059 | T | C | - | 4.36 |
| NS1 | 2451 | T | C | - | 4.18 | NS4B | 7062 | G | A | - | 4.33 |
| NS1 | 2484 | T | C | - | 3.13 | NS4B | 7101 | C | T | - | 15.31 |
| NS1 | 2511 | G | A | - | 5.22 | NS4B | 7134 | C | T | - | 2.17 |
| NS1 | 2589 | A | G | - | 4.68 | NS4B | 7137 | T | C | - | 4.47 |
| NS1 | 2781 | G | A | - | 4.12 | NS4B | 7161 | C | T | - | 5.90 |
| NS1 | 2911 | A | T | Thr164Ser | 2.47 | NS4B | 7165 | T | C | - | 4.25 |
| NS1 | 2991 | C | T | - | 2.62 | NS4B | 7248 | C | T | - | 2.91 |
| NS1 | 3025 | G | A | Val202Ile | 6.34 | NS4B | 7356 | C | T | - | 3.83 |
| NS1 | 3152 | C | CA | Asn246fs | 3.48 | NS4B | 7383 | T | C | - | 3.96 |
| NS1 | 3234 | T | C | - | 2.20 | NS4B | 7393 | C | T | - | 7.60 |
| NS1 | 3330 | C | T | - | 2.27 | NS4B | 7428 | C | T | - | 1.98 |
| NS1 | 3402 | T | C | - | 4.74 | NS4B | 7458 | A | G | - | 9.38 |
| NS1 | 3432 | T | C | - | 6.63 | NS4B | 7518 | C | T | - | 13.85 |
| NS1 | 3438 | A | G | - | 6.52 | NS5 | 7582 | G | A | Val5Ile | 6.67 |
| NS1 | 3447 | A | G | - | 8.05 | NS5 | 7776 | C | T | - | 11.32 |
| NS1 | 3462 | T | C | - | 17.14 | NS5 | 7812 | T | C | - | 14.00 |
| NS1 | 3474 | A | G | - | 12.00 | NS5 | 7911 | C | T | - | 3.73 |
| NS2A | 3489 | G | A | - | 7.55 | NS5 | 7959 | C | T | - | 4.13 |
| NS2A | 3514 | C | T | - | 36.96 | NS5 | 8049 | A | G | - | 3.53 |
| NS2A | 3552 | C | T | - | 41.18 | NS5 | 8083 | C | T | - | 2.89 |
| NS2A | 3597 | C | T | - | 17.57 | NS5 | 8284 | T | C | - | 2.83 |
| NS2A | 3612 | T | A | - | 3.38 | NS5 | 8298 | C | T | - | 2.66 |
| NS2A | 3624 | T | C | - | 10.68 | NS5 | 8310 | T | C | - | 5.41 |
| NS2A | 3629 | G | A | Arg51Lys | 9.68 | NS5 | 8319 | C | T | - | 2.23 |
| NS2A | 3743 | C | T | Ala89Val | 1.86 | NS5 | 8469 | C | T | - | 7.07 |
| NS2A | 3750 | A | G | - | 1.38 | NS5 | 8544 | C | T | - | 3.35 |
| NS2A | 3753 | C | T | - | 3.77 | NS5 | 8572 | A | G | Ile335Val | 2.44 |
| NS2A | 3768 | C | T | - | 4.07 | NS5 | 8810 | C | T | Thr414Ile | 2.94 |
| NS2A | 3813 | T | C | - | 6.87 | NS5 | 8967 | C | T | - | 6.52 |
| NS2A | 3858 | A | G | - | 1.92 | NS5 | 9042 | T | C | - | 3.98 |
| NS2A | 3864 | G | A | - | 3.88 | NS5 | 9066 | C | T | - | 6.63 |
| NS2A | 3875 | C | T | Ala133Val | 4.48 | NS5 | 9079 | C | T | - | 6.14 |
| NS2A | 3879 | C | A | - | 3.95 | NS5 | 9112 | C | T | - | 6.41 |
| NS2A | 3906 | C | T | - | 1.60 | NS5 | 9294 | C | T | - | 12.50 |
| NS2A | 3921 | T | C | - | 4.72 | NS5 | 9501 | G | C | Gln644His | 1.59 |
| NS2A | 4011 | T | C | - | 3.08 | NS5 | 9628 | G | A | Val687Ile | 1.43 |

| | | | | | |
|---|---|---|---|---|---|
| NS2A | 4034 | A | G | Gln186Arg | 3.41 |
| NS2A | 4063 | C | T | - | 3.49 |
| NS2A | 4083 | C | T | - | 3.82 |
| NS2A | 4092 | T | C | - | 7.04 |
| NS2A | 4110 | T | C | - | 3.24 |
| NS2B | 4164 | C | T | - | 3.99 |
| NS2B | 4206 | T | C | - | 4.17 |
| NS2B | 4212 | C | T | - | 5.74 |
| NS2B | 4278 | C | T | - | 5.26 |
| NS2B | 4287 | G | A | - | 6.83 |
| NS2B | 4329 | A | G | - | 6.18 |
| NS2B | 4338 | A | G | - | 3.24 |
| NS5 | 9795 | G | A | - | 1.66 |
| NS5 | 9825 | T | C | - | 2.65 |
| NS5 | 10071 | A | T | Glu834Asp | 1.92 |
| NS5 | 10083 | C | T | - | 2.83 |
| NS5 | 10155 | T | C | - | 5.12 |
| NS5 | 10202 | G | A | Gly878Glu | 9.65 |
| NS5 | 10203 | A | G | - | 3.54 |
| NS5 | 10252 | G | A | Glu895Lys | 7.69 |
| 3UTR | 10283 | C | T | - | 8.82 |
| 3UTR | 10389 | C | CA | - | 1.41 |
| 3UTR | 10407 | T | C | - | 2.10 |
| 3UTR | 10443 | T | TA | - | 2.54 |

| **204** | | | | | |
|---|---|---|---|---|---|
| SD | | | | | |
| Primary | | | | | |
| 6.75E+03 | | | | | |
| 8 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| 5UTR | 70 | G | GT | - | 6.67 |
| C | 111 | A | G | - | 9.38 |
| C | 201 | T | C | - | 19.10 |
| C | 315 | G | A | - | 15.71 |
| C | 384 | C | T | - | 20.78 |
| C | 387 | G | A | - | 20.00 |
| C | 393 | T | C | - | 6.45 |
| C | 431 | T | C | Val112Ala | 29.25 |
| prM/M | 445 | T | C | - | 23.53 |
| prM/M | 450 | C | T | - | 14.58 |
| prM/M | 508 | C | T | - | 24.76 |
| prM/M | 523 | A | G | Asn29Asp | 26.36 |
| prM/M | 525 | C | T | - | 26.42 |
| prM/M | 531 | T | C | - | 44.95 |
| prM/M | 567 | G | A | - | 26.00 |
| prM/M | 606 | C | T | - | 12.50 |
| prM/M | 651 | G | A | - | 12.50 |
| prM/M | 703 | G | GA | Arg91fs | 9.52 |
| prM/M | 717 | A | G | - | 8.51 |
| prM/M | 792 | G | A | - | 25.49 |
| prM/M | 798 | T | C | - | 22.86 |
| prM/M | 834 | T | C | - | 12.73 |
| prM/M | 837 | T | C | - | 14.15 |
| prM/M | 852 | G | A | - | 21.21 |
| prM/M | 856 | C | T | - | 19.23 |
| prM/M | 864 | C | T | - | 19.17 |
| prM/M | 873 | A | C | - | 18.38 |
| prM/M | 894 | C | A | - | 16.94 |
| E | 954 | A | C | - | 18.55 |
| E | 1005 | C | A | - | 16.19 |
| E | 1023 | T | C | - | 39.47 |
| E | 1094 | C | T | Pro53Leu | 9.52 |
| E | 1102 | C | T | - | 14.06 |
| E | 1214 | A | G | Lys93Arg | 15.56 |
| E | 1287 | T | C | - | 12.90 |
| E | 1293 | T | C | - | 22.58 |
| E | 1396 | G | A | Asp154Asn | 15.91 |
| E | 1413 | A | C | - | 42.50 |
| E | 1473 | C | T | - | 19.12 |
| E | 1545 | T | C | - | 24.30 |
| NS3 | 4596 | C | T | - | 22.45 |
| NS3 | 4746 | T | C | - | 20.45 |
| NS3 | 4812 | C | T | - | 8.97 |
| NS3 | 4829 | G | GA | Asn105fs | 5.26 |
| NS3 | 4830 | A | G | - | 17.11 |
| NS3 | 4905 | G | A | - | 25.96 |
| NS3 | 4944 | C | T | - | 24.72 |
| NS3 | 4959 | T | C | - | 27.06 |
| NS3 | 4971 | C | T | - | 32.56 |
| NS3 | 4974 | T | C | - | 7.78 |
| NS3 | 5004 | C | T | - | 18.68 |
| NS3 | 5070 | C | T | - | 19.10 |
| NS3 | 5082 | A | G | - | 41.86 |
| NS3 | 5190 | C | T | - | 10.16 |
| NS3 | 5277 | C | T | - | 30.00 |
| NS3 | 5283 | A | G | - | 32.76 |
| NS3 | 5304 | T | C | - | 13.46 |
| NS3 | 5451 | C | A | - | 36.36 |
| NS3 | 5460 | T | C | - | 37.78 |
| NS3 | 5583 | T | C | - | 8.20 |
| NS3 | 5610 | A | G | - | 9.62 |
| NS3 | 5613 | T | C | - | 15.25 |
| NS3 | 5631 | C | T | - | 7.02 |
| NS3 | 5649 | G | A | - | 12.00 |
| NS3 | 5730 | C | T | - | 17.65 |
| NS3 | 5802 | C | T | - | 38.46 |
| NS3 | 5835 | A | G | - | 18.18 |
| NS3 | 5844 | G | A | - | 17.78 |
| NS3 | 5847 | C | T | - | 43.90 |
| NS3 | 5859 | C | T | - | 33.33 |
| NS3 | 5915 | C | CA | Asn467fs | 9.76 |
| NS3 | 6009 | T | C | - | 20.63 |
| NS3 | 6033 | T | C | - | 29.69 |
| NS3 | 6069 | G | T | - | 26.53 |
| NS3 | 6094 | T | C | - | 13.85 |
| NS3 | 6099 | A | G | - | 16.18 |
| NS3 | 6157 | T | C | - | 46.38 |
| NS3 | 6159 | G | A | - | 35.71 |
| NS3 | 6220 | A | G | Ile567Val | 20.00 |
| NS3 | 6222 | T | C | - | 11.25 |
| NS3 | 6285 | G | A | - | 5.88 |
| NS3 | 6300 | C | T | - | 22.67 |
| NS4A | 6378 | T | C | - | 18.63 |
| NS4A | 6379 | T | C | - | 18.45 |
| NS4A | 6390 | C | T | - | 4.42 |
| NS4A | 6462 | T | C | - | 14.53 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1590 | G | A | - | 12.60 | NS4A | 6471 | T | C | - | 8.33 |
| E | 1599 | G | A | - | 15.65 | NS4A | 6491 | A | G | Lys39Arg | 10.17 |
| E | 1626 | C | T | - | 13.13 | NS4A | 6496 | T | C | Tyr41His | 11.29 |
| E | 1641 | G | A | - | 16.85 | NS4A | 6500 | C | A | Thr42Asn | 9.30 |
| E | 1710 | G | T | - | 5.95 | NS4A | 6512 | A | G | Asn46Ser | 24.22 |
| E | 1842 | T | C | - | 22.54 | NS4A | 6528 | T | C | - | 10.61 |
| E | 1863 | G | A | - | 12.96 | NS4A | 6547 | C | T | - | 11.68 |
| E | 1944 | C | T | - | 21.15 | NS4A | 6556 | C | T | - | 15.50 |
| E | 1959 | T | C | - | 37.25 | NS4A | 6562 | G | A | Ala63Thr | 16.81 |
| E | 1972 | T | C | Tyr346His | 25.42 | NS4A | 6585 | A | G | - | 11.19 |
| E | 1995 | A | G | - | 20.00 | NS4A | 6633 | T | C | - | 31.73 |
| E | 2023 | A | G | Ser363Gly | 23.19 | NS4A | 6654 | T | C | - | 28.57 |
| E | 2024 | G | C | | 24.64 | NS4A | 6681 | A | G | - | 21.79 |
| E | 2049 | C | T | - | 36.73 | NS4B | 6841 | C | T | - | 22.12 |
| E | 2055 | C | T | - | 15.09 | NS4B | 6864 | C | T | - | 8.33 |
| E | 2067 | C | T | - | 36.96 | NS4B | 6871 | G | A | Gly16Arg | 10.43 |
| E | 2073 | C | T | - | 46.67 | NS4B | 6879 | T | C | - | 19.33 |
| E | 2223 | T | C | - | 7.96 | NS4B | 6948 | T | C | - | 27.27 |
| E | 2247 | T | C | - | 21.31 | NS4B | 6957 | C | T | - | 29.76 |
| E | 2276 | C | T | Ala447Val | 9.79 | NS4B | 7008 | A | G | - | 26.89 |
| E | 2289 | C | T | - | 13.29 | NS4B | 7018 | T | C | - | 25.00 |
| E | 2307 | C | T | - | 15.07 | NS4B | 7038 | A | G | - | 11.66 |
| E | 2319 | C | A | - | 11.81 | NS4B | 7041 | T | C | - | 24.26 |
| E | 2376 | G | A | - | 20.17 | NS4B | 7059 | T | C | - | 14.07 |
| NS1 | 2434 | A | G | Ile5Val | 25.00 | NS4B | 7062 | G | A | - | 23.91 |
| NS1 | 2448 | A | G | - | 12.87 | NS4B | 7083 | G | A | - | 5.00 |
| NS1 | 2451 | T | C | - | 22.45 | NS4B | 7137 | T | C | - | 16.46 |
| NS1 | 2484 | T | C | - | 21.57 | NS4B | 7165 | T | C | - | 25.00 |
| NS1 | 2511 | G | A | - | 32.98 | NS4B | 7186 | G | A | Val121Ile | 33.87 |
| NS1 | 2589 | A | G | - | 36.36 | NS4B | 7248 | C | T | - | 16.00 |
| NS1 | 2685 | T | C | - | 13.92 | NS4B | 7314 | A | G | - | 17.24 |
| NS1 | 2697 | C | T | - | 15.94 | NS4B | 7356 | C | T | - | 20.43 |
| NS1 | 2731 | T | C | - | 17.65 | NS4B | 7383 | T | C | - | 26.25 |
| NS1 | 2736 | G | C | - | 22.00 | NS4B | 7410 | T | C | - | 37.50 |
| NS1 | 2781 | G | A | - | 29.17 | NS4B | 7428 | C | T | - | 33.72 |
| NS1 | 2835 | T | C | - | 5.80 | NS4B | 7518 | T | C | - | 40.48 |
| NS1 | 2850 | C | T | - | 6.56 | NS5 | 7758 | A | G | - | 21.88 |
| NS1 | 2898 | T | C | - | 12.90 | NS5 | 7767 | C | T | - | 8.82 |
| NS1 | 2961 | C | T | - | 15.66 | NS5 | 7776 | C | T | - | 12.12 |
| NS1 | 2966 | A | G | Lys182Arg | 4.60 | NS5 | 7794 | A | G | - | 37.93 |
| NS1 | 2976 | A | G | - | 18.52 | NS5 | 7812 | T | C | - | 25.00 |
| NS1 | 2989 | A | G | Asn190Asp | 21.35 | NS5 | 7911 | C | T | - | 37.80 |
| NS1 | 3009 | T | C | - | 22.00 | NS5 | 7959 | C | T | - | 33.33 |
| NS1 | 3075 | T | C | - | 21.25 | NS5 | 7999 | T | C | - | 14.47 |
| NS1 | 3081 | A | T | - | 23.68 | NS5 | 8049 | A | G | - | 23.24 |
| NS1 | 3114 | C | A | - | 6.76 | NS5 | 8083 | C | T | - | 18.03 |
| NS1 | 3152 | C | CA | Asn246fs | 4.23 | NS5 | 8121 | T | C | - | 12.73 |
| NS1 | 3234 | T | C | - | 21.50 | NS5 | 8139 | C | T | - | 6.90 |
| NS1 | 3291 | C | T | - | 28.78 | NS5 | 8184 | A | G | - | 7.29 |
| NS1 | 3330 | C | T | - | 11.59 | NS5 | 8214 | G | A | - | 6.48 |
| NS1 | 3342 | C | T | - | 5.88 | NS5 | 8241 | T | C | - | 27.08 |
| NS1 | 3402 | T | C | - | 20.00 | NS5 | 8284 | T | C | - | 18.07 |
| NS1 | 3432 | T | C | - | 24.42 | NS5 | 8310 | T | C | - | 18.00 |
| NS1 | 3438 | A | G | - | 26.51 | NS5 | 8380 | G | A | Val271Ile | 45.45 |
| NS1 | 3447 | A | G | - | 28.38 | NS5 | 8469 | C | T | - | 33.33 |
| NS1 | 3474 | G | A | - | 24.49 | NS5 | 8544 | C | T | - | 34.43 |
| NS2A | 3489 | A | G | - | 47.37 | NS5 | 8569 | G | A | Val334Ile | 7.58 |
| NS2A | 3552 | T | C | - | 29.09 | NS5 | 8574 | C | T | - | 7.46 |
| NS2A | 3595 | T | C | Phe40Leu | 6.02 | NS5 | 8604 | C | T | - | 14.29 |
| NS2A | 3597 | C | T | - | 47.50 | NS5 | 8628 | T | C | - | 40.00 |

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|---|---|---|---|---|---|
| NS2A | 3604 | T | C | - | 27.27 |
| NS2A | 3624 | T | C | - | 28.26 |
| NS2A | 3629 | G | A | Arg51Lys | 29.35 |
| NS2A | 3663 | C | T | - | 33.33 |
| NS2A | 3750 | A | G | - | 9.27 |
| NS2A | 3753 | C | T | - | 18.27 |
| NS2A | 3768 | C | T | - | 18.41 |
| NS2A | 3801 | C | T | - | 5.96 |
| NS2A | 3858 | A | G | - | 4.98 |
| NS2A | 3864 | G | A | - | 18.08 |
| NS2A | 3875 | C | T | Ala133Val | 15.38 |
| NS2A | 3876 | C | T | - | 3.24 |
| NS2A | 3879 | C | A | - | 13.65 |
| NS2A | 3910 | T | C | - | 7.37 |
| NS2A | 3921 | T | C | - | 20.13 |
| NS2A | 4011 | T | C | - | 22.87 |
| NS2A | 4034 | A | G | Gln186Arg | 8.61 |
| NS2A | 4059 | G | A | - | 26.44 |
| NS2A | 4083 | C | T | - | 27.43 |
| NS2A | 4110 | T | C | - | 7.39 |
| NS2B | 4164 | C | T | - | 27.17 |
| NS2B | 4249 | G | A | Val40Ile | 11.54 |
| NS2B | 4257 | C | T | - | 7.96 |
| NS2B | 4278 | C | T | - | 40.43 |
| NS2B | 4329 | A | G | - | 30.91 |
| NS2B | 4338 | A | G | - | 20.45 |
| NS2B | 4344 | C | T | - | 15.91 |
| NS2B | 4350 | T | C | - | 13.04 |
| NS2B | 4414 | C | T | - | 10.87 |
| NS2B | 4438 | C | T | - | 25.81 |
| NS2B | 4482 | G | A | - | 17.86 |
| NS2B | 4491 | A | G | - | 29.82 |
| NS2B | 4509 | G | T | - | 32.08 |
| NS5 | 8658 | T | C | - | 33.33 |
| NS5 | 8803 | G | A | Val412Ile | 25.00 |
| NS5 | 8859 | G | T | Arg430Ser | 43.48 |
| NS5 | 8967 | C | T | - | 37.14 |
| NS5 | 8979 | C | T | - | 35.71 |
| NS5 | 9012 | G | A | - | 18.18 |
| NS5 | 9066 | C | T | - | 18.37 |
| NS5 | 9079 | C | T | - | 17.31 |
| NS5 | 9112 | C | T | - | 37.50 |
| NS5 | 9120 | T | C | - | 42.11 |
| NS5 | 9132 | A | G | - | 30.00 |
| NS5 | 9369 | C | A | - | 25.00 |
| NS5 | 9375 | G | A | - | 21.43 |
| NS5 | 9399 | T | C | - | 17.86 |
| NS5 | 9402 | T | C | - | 13.33 |
| NS5 | 9424 | T | C | - | 14.71 |
| NS5 | 9501 | G | C | Gln644His | 27.59 |
| NS5 | 9576 | T | C | - | 9.62 |
| NS5 | 9795 | G | A | - | 11.35 |
| NS5 | 9825 | T | C | - | 15.97 |
| NS5 | 9888 | G | A | - | 30.08 |
| NS5 | 9942 | T | C | - | 7.81 |
| NS5 | 10075 | A | G | Ile836Val | 21.19 |
| NS5 | 10083 | C | T | - | 17.19 |
| NS5 | 10089 | G | A | - | 28.70 |
| NS5 | 10155 | C | T | - | 48.57 |
| NS5 | 10158 | C | T | - | 14.08 |
| NS5 | 10202 | A | G | Glu878Gly | 38.46 |
| NS5 | 10252 | A | G | Lys895Glu | 43.75 |
| 3UTR | 10389 | C | CA | - | 4.31 |
| 3UTR | 10389 | C | CAA | - | 2.59 |
| 3UTR | 10407 | T | C | - | 11.38 |
| 3UTR | 10443 | T | TA | - | 3.10 |

| **205** | | | | | |
|---|---|---|---|---|---|
| WS | | | | | |
| Secondary | | | | | |
| 3.85E+01 | | | | | |
| 1 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 111 | G | A | - | 29.17 |
| C | 181 | C | T | - | 8.11 |
| C | 201 | T | C | - | 8.75 |
| C | 213 | A | G | - | 8.43 |
| C | 219 | C | A | - | 8.89 |
| C | 228 | A | G | - | 11.34 |
| C | 231 | A | G | - | 4.95 |
| C | 246 | T | G | - | 11.76 |
| C | 252 | G | A | - | 11.69 |
| C | 315 | G | A | - | 11.48 |
| C | 327 | T | C | - | 12.70 |
| C | 345 | G | C | - | 5.41 |
| C | 348 | C | T | - | 5.33 |
| C | 375 | C | T | - | 6.56 |
| C | 384 | C | T | - | 20.69 |
| C | 387 | G | A | - | 14.04 |
| C | 431 | T | C | Val112Ala | 26.92 |
| prM/M | 445 | T | C | - | 13.19 |
| prM/M | 450 | C | T | - | 10.99 |
| NS2B | 4401 | G | A | - | 23.53 |
| NS2B | 4414 | C | T | - | 22.22 |
| NS2B | 4438 | T | C | - | 38.89 |
| NS2B | 4487 | C | T | Ala119Val | 12.50 |
| NS2B | 4491 | A | G | - | 17.65 |
| NS2B | 4509 | G | T | - | 16.67 |
| NS3 | 4530 | A | G | - | 6.90 |
| NS3 | 4539 | C | T | - | 8.00 |
| NS3 | 4557 | A | C | - | 7.55 |
| NS3 | 4566 | A | G | - | 14.00 |
| NS3 | 4573 | C | T | - | 15.56 |
| NS3 | 4596 | C | T | - | 19.15 |
| NS3 | 4602 | A | G | - | 37.78 |
| NS3 | 4604 | G | A | Arg28Lys | 37.78 |
| NS3 | 4620 | T | C | - | 43.48 |
| NS3 | 4623 | T | C | - | 45.45 |
| NS3 | 4629 | C | T | - | 40.54 |
| NS3 | 4635 | T | C | - | 43.18 |
| NS3 | 4641 | C | T | - | 44.74 |
| NS3 | 4662 | T | C | - | 50.00 |
| NS3 | 4695 | A | G | - | 46.15 |
| NS3 | 4703 | A | G | Lys61Arg | 46.88 |
| NS3 | 4731 | A | G | - | 30.77 |
| NS3 | 4739 | A | G | Lys73Arg | 23.81 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| prM/M | 480 | T | C | - | 5.22 | NS3 | 4746 | T | C | - | 27.78 |
| prM/M | 481 | G | A | Gly15Ser | 5.22 | NS3 | 4806 | C | T | - | 7.50 |
| prM/M | 507 | T | C | - | 8.16 | NS3 | 4812 | C | T | - | 19.15 |
| prM/M | 508 | C | T | - | 24.49 | NS3 | 4824 | A | G | - | 7.69 |
| prM/M | 513 | C | T | - | 7.92 | NS3 | 4830 | A | G | - | 12.73 |
| prM/M | 523 | A | G | Asn29Asp | 25.49 | NS3 | 4833 | A | G | - | 7.27 |
| prM/M | 525 | C | T | - | 26.00 | NS3 | 4854 | G | A | - | 6.58 |
| prM/M | 531 | C | T | - | 19.13 | NS3 | 4905 | G | A | - | 20.93 |
| prM/M | 531 | C | G | - | 9.57 | NS3 | 4938 | T | C | - | 7.95 |
| prM/M | 555 | A | G | Ile39Met | 6.36 | NS3 | 4944 | C | T | - | 12.36 |
| prM/M | 567 | G | A | - | 13.40 | NS3 | 4946 | G | A | Arg142Lys | 7.78 |
| prM/M | 651 | G | A | - | 15.15 | NS3 | 4959 | T | C | - | 24.72 |
| prM/M | 792 | G | A | - | 17.39 | NS3 | 4971 | C | T | - | 25.29 |
| prM/M | 798 | T | C | - | 14.74 | NS3 | 4974 | T | C | - | 10.14 |
| prM/M | 834 | T | C | - | 8.47 | NS3 | 5004 | T | C | - | 21.33 |
| prM/M | 837 | T | C | - | 8.85 | NS3 | 5026 | G | GA | Ser171fs | 5.41 |
| prM/M | 852 | G | A | - | 21.57 | NS3 | 5070 | C | T | - | 13.04 |
| prM/M | 856 | C | T | - | 20.75 | NS3 | 5082 | G | A | - | 28.77 |
| prM/M | 864 | C | T | - | 7.56 | NS3 | 5130 | C | T | - | 6.59 |
| prM/M | 873 | A | C | - | 10.94 | NS3 | 5139 | C | A | - | 6.59 |
| prM/M | 876 | G | A | - | 14.40 | NS3 | 5145 | T | C | - | 6.00 |
| prM/M | 880 | C | T | His148Tyr | 13.85 | NS3 | 5151 | A | G | - | 10.47 |
| prM/M | 882 | C | T | - | 13.04 | NS3 | 5163 | T | A | - | 8.51 |
| prM/M | 891 | G | A | - | 14.73 | NS3 | 5182 | C | T | - | 4.00 |
| prM/M | 893 | C | T | Ala152Val | 14.62 | NS3 | 5190 | C | T | - | 7.21 |
| prM/M | 894 | C | A | - | 16.15 | NS3 | 5235 | T | C | - | 16.49 |
| prM/M | 921 | C | T | - | 2.99 | NS3 | 5277 | C | T | - | 26.09 |
| E | 954 | A | C | - | 5.69 | NS3 | 5283 | A | G | - | 20.93 |
| E | 960 | T | C | - | 14.29 | NS3 | 5286 | G | A | - | 8.70 |
| E | 969 | C | T | - | 12.17 | NS3 | 5319 | T | C | - | 38.10 |
| E | 972 | A | G | - | 12.61 | NS3 | 5340 | C | T | - | 27.27 |
| E | 1005 | C | A | - | 23.89 | NS3 | 5451 | C | A | - | 26.47 |
| E | 1023 | C | T | - | 22.50 | NS3 | 5451 | C | T | - | 11.76 |
| E | 1032 | G | A | - | 13.39 | NS3 | 5460 | T | C | - | 14.29 |
| E | 1040 | C | CA | Asn37fs | 5.41 | NS3 | 5508 | T | C | - | 21.28 |
| E | 1062 | T | C | - | 15.52 | NS3 | 5532 | A | G | - | 31.82 |
| E | 1068 | A | G | - | 14.85 | NS3 | 5544 | G | A | - | 39.13 |
| E | 1089 | A | G | - | 15.58 | NS3 | 5550 | A | G | - | 34.00 |
| E | 1094 | C | T | Pro53Leu | 8.33 | NS3 | 5559 | A | T | - | 32.08 |
| E | 1102 | C | T | - | 10.29 | NS3 | 5574 | C | T | - | 29.63 |
| E | 1122 | A | G | - | 25.00 | NS3 | 5583 | T | C | - | 28.30 |
| E | 1148 | A | C | Glu71Ala | 15.38 | NS3 | 5610 | A | G | - | 15.79 |
| E | 1152 | G | T | - | 15.38 | NS3 | 5613 | T | C | - | 33.33 |
| E | 1155 | T | A | - | 19.05 | NS3 | 5631 | C | T | - | 10.81 |
| E | 1214 | A | G | Lys93Arg | 21.43 | NS3 | 5730 | C | T | - | 34.78 |
| E | 1266 | G | A | - | 8.33 | NS3 | 5766 | C | T | - | 10.26 |
| E | 1287 | T | C | - | 14.71 | NS3 | 5775 | G | A | - | 14.29 |
| E | 1293 | T | C | - | 24.64 | NS3 | 5793 | C | T | - | 14.89 |
| E | 1302 | G | A | - | 8.57 | NS3 | 5802 | C | T | - | 31.58 |
| E | 1317 | A | G | - | 9.38 | NS3 | 5817 | T | C | - | 9.30 |
| E | 1326 | A | G | - | 6.06 | NS3 | 5823 | A | G | - | 15.56 |
| E | 1329 | G | A | - | 6.25 | NS3 | 5829 | T | C | - | 10.64 |
| E | 1396 | G | A | Asp154Asn | 31.58 | NS3 | 5832 | C | T | - | 10.64 |
| E | 1413 | A | C | - | 29.41 | NS3 | 5835 | A | G | - | 10.64 |
| E | 1473 | C | T | - | 36.59 | NS3 | 5841 | G | A | - | 11.90 |
| E | 1536 | G | A | - | 3.92 | NS3 | 5847 | C | T | - | 24.39 |
| E | 1543 | G | A | Asp203Asn | 4.67 | NS3 | 5859 | C | T | - | 30.30 |
| E | 1545 | T | C | - | 33.65 | NS3 | 5868 | G | A | - | 16.00 |
| E | 1555 | C | T | - | 4.81 | NS3 | 5886 | G | A | - | 16.00 |
| E | 1576 | C | T | - | 6.42 | NS3 | 5910 | A | G | - | 18.18 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1590 | G | A | - | 23.33 | NS3 | 5934 | G | A | - | 15.00 |
| E | 1599 | G | A | - | 25.27 | NS3 | 6009 | T | C | - | 11.11 |
| E | 1611 | T | C | - | 7.53 | NS3 | 6033 | T | C | - | 32.50 |
| E | 1626 | C | T | - | 33.33 | NS3 | 6063 | A | G | - | 16.00 |
| E | 1638 | A | G | - | 8.70 | NS3 | 6066 | A | G | - | 16.67 |
| E | 1641 | G | A | - | 14.13 | NS3 | 6087 | A | G | - | 20.00 |
| E | 1653 | T | C | - | 9.30 | NS3 | 6093 | T | C | - | 16.95 |
| E | 1668 | C | T | - | 8.54 | NS3 | 6094 | T | C | - | 10.34 |
| E | 1671 | C | A | - | 9.30 | NS3 | 6096 | G | A | - | 17.86 |
| E | 1686 | C | T | - | 12.16 | NS3 | 6099 | A | G | - | 8.62 |
| E | 1692 | C | T | - | 11.11 | NS3 | 6108 | A | G | - | 28.07 |
| E | 1698 | G | A | - | 9.09 | NS3 | 6141 | G | A | - | 24.62 |
| E | 1713 | C | T | - | 11.43 | NS3 | 6157 | C | T | - | 30.36 |
| E | 1719 | C | T | - | 7.94 | NS3 | 6159 | G | A | - | 36.54 |
| E | 1722 | G | A | - | 9.38 | NS3 | 6167 | A | G | Lys549Arg | 30.77 |
| E | 1728 | C | T | - | 10.77 | NS3 | 6177 | T | C | - | 30.19 |
| E | 1765 | T | C | - | 13.85 | NS3 | 6186 | C | T | - | 26.92 |
| E | 1776 | G | A | - | 15.94 | NS3 | 6192 | C | T | - | 28.00 |
| E | 1842 | T | C | - | 8.33 | NS3 | 6203 | A | G | Lys588Arg | 27.66 |
| E | 1858 | A | G | Ile308Val | 12.50 | NS3 | 6220 | A | G | Ile567Val | 34.88 |
| E | 1860 | C | T | - | 13.16 | NS3 | 6222 | T | C | - | 18.18 |
| E | 1863 | G | A | - | 28.95 | NS3 | 6237 | A | C | - | 8.51 |
| E | 1920 | G | T | - | 18.18 | NS3 | 6300 | C | T | - | 7.55 |
| E | 1923 | C | T | - | 15.38 | NS3 | 6303 | A | G | - | 5.56 |
| E | 1938 | G | A | - | 17.95 | NS3 | 6312 | T | C | - | 5.56 |
| E | 1944 | C | T | - | 50.00 | NS3 | 6333 | A | G | - | 15.66 |
| E | 1950 | G | A | - | 14.29 | NS3 | 6339 | A | G | - | 14.74 |
| E | 1955 | C | T | Thr340Ile | 16.28 | NS3 | 6342 | A | C | - | 13.46 |
| E | 1956 | A | G | - | 16.28 | NS3 | 6354 | G | A | - | 12.15 |
| E | 1959 | C | T | - | 32.56 | NS3 | 6357 | A | G | - | 11.93 |
| E | 1960 | T | C | - | 13.64 | NS3 | 6366 | T | C | - | 14.29 |
| E | 1972 | C | T | His346Tyr | 45.65 | NS4A | 6378 | T | C | - | 32.04 |
| E | 1974 | C | T | - | 16.36 | NS4A | 6379 | T | C | - | 32.67 |
| E | 1983 | A | T | - | 18.97 | NS4A | 6390 | C | T | - | 18.97 |
| E | 1995 | A | G | - | 25.00 | NS4A | 6423 | C | T | - | 22.89 |
| E | 1998 | T | C | - | 20.37 | NS4A | 6429 | T | C | - | 18.07 |
| E | 2007 | C | T | - | 30.91 | NS4A | 6435 | G | A | - | 15.12 |
| E | 2010 | A | C | - | 22.22 | NS4A | 6441 | A | G | - | 12.09 |
| E | 2022 | T | C | - | 19.61 | NS4A | 6444 | C | T | - | 12.50 |
| E | 2023 | A | G | Ser363Gly | 22.92 | NS4A | 6448 | C | T | - | 11.96 |
| E | 2024 | G | C | | 21.15 | NS4A | 6462 | T | C | - | 16.47 |
| E | 2049 | T | C | - | 47.83 | NS4A | 6466 | C | T | - | 12.24 |
| E | 2055 | C | T | - | 19.57 | NS4A | 6471 | T | C | - | 7.21 |
| E | 2067 | C | T | - | 44.00 | NS4A | 6491 | A | G | Lys39Arg | 10.00 |
| E | 2073 | T | C | - | 28.26 | NS4A | 6496 | T | C | Tyr41His | 6.84 |
| E | 2074 | G | A | Val380Ile | 13.64 | NS4A | 6500 | C | A | Thr42Asn | 6.98 |
| E | 2085 | G | A | - | 13.16 | NS4A | 6512 | A | G | Asn46Ser | 19.09 |
| E | 2118 | G | A | - | 12.12 | NS4A | 6528 | T | C | - | 15.93 |
| E | 2124 | T | C | - | 12.90 | NS4A | 6538 | C | T | - | 4.13 |
| E | 2127 | C | A | - | 12.90 | NS4A | 6546 | A | G | - | 3.97 |
| E | 2142 | T | C | - | 13.33 | NS4A | 6547 | C | T | - | 10.66 |
| E | 2163 | A | G | - | 24.14 | NS4A | 6556 | C | T | - | 12.30 |
| E | 2175 | C | T | - | 18.42 | NS4A | 6562 | G | A | Ala63Thr | 12.61 |
| E | 2223 | T | C | - | 19.79 | NS4A | 6570 | A | G | - | 8.06 |
| E | 2241 | T | C | - | 11.65 | NS4A | 6573 | A | T | - | 8.00 |
| E | 2247 | T | C | - | 18.37 | NS4A | 6582 | C | T | - | 9.84 |
| E | 2250 | A | G | - | 10.31 | NS4A | 6585 | A | G | - | 11.57 |
| E | 2256 | C | T | - | 12.77 | NS4A | 6588 | C | T | - | 9.76 |
| E | 2276 | C | T | Ala447Val | 4.42 | NS4A | 6591 | A | G | - | 10.26 |
| E | 2277 | T | C | - | 11.71 | NS4A | 6615 | G | A | - | 15.73 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 2280 | T | C | - | 11.82 | NS4A | 6633 | T | C | - | 18.99 |
| E | 2283 | T | C | - | 11.82 | NS4A | 6654 | T | C | - | 16.67 |
| E | 2286 | G | A | - | 12.62 | NS4A | 6678 | A | G | - | 13.21 |
| E | 2289 | C | T | - | 7.75 | NS4A | 6693 | A | G | - | 15.94 |
| E | 2298 | T | C | - | 12.90 | NS4A | 6732 | C | T | - | 16.42 |
| E | 2307 | C | T | - | 8.00 | NS4A | 6777 | G | A | - | 26.98 |
| E | 2319 | C | A | - | 4.38 | NS4A | 6780 | C | T | - | 28.13 |
| E | 2319 | C | T | - | 14.60 | NS4B | 6837 | T | G | - | 29.21 |
| E | 2320 | A | G | Ile462Val | 14.49 | NS4B | 6840 | C | T | - | 29.55 |
| E | 2328 | A | T | - | 4.76 | NS4B | 6841 | C | T | - | 11.36 |
| E | 2349 | T | C | - | 14.58 | NS4B | 6852 | C | G | - | 27.27 |
| E | 2367 | G | A | - | 10.26 | NS4B | 6861 | C | T | - | 22.86 |
| E | 2371 | C | T | - | 10.43 | NS4B | 6862 | C | T | Leu13Phe | 21.90 |
| E | 2376 | G | A | - | 28.44 | NS4B | 6864 | C | T | - | 3.77 |
| E | 2379 | G | A | - | 7.08 | NS4B | 6870 | T | G | Phe15Leu | 26.80 |
| E | 2395 | C | T | - | 8.65 | NS4B | 6879 | T | C | - | 9.71 |
| E | 2400 | C | T | - | 5.43 | NS4B | 6880 | A | G | Thr19Ala | 21.21 |
| E | 2401 | C | T | - | 5.88 | NS4B | 6889 | G | C | Glu22Gln | 12.87 |
| E | 2408 | C | T | Ala491Val | 6.41 | NS4B | 6891 | A | G | - | 14.14 |
| NS1 | 2433 | C | T | - | 10.98 | NS4B | 6892 | T | C | Ser23Pro | 13.54 |
| NS1 | 2434 | A | G | Ile5Val | 28.05 | NS4B | 6894 | T | C | - | 16.30 |
| NS1 | 2442 | C | T | - | 12.79 | NS4B | 6924 | T | C | - | 11.03 |
| NS1 | 2448 | A | G | - | 10.13 | NS4B | 6942 | G | C | - | 11.97 |
| NS1 | 2451 | T | C | - | 33.75 | NS4B | 6948 | T | C | - | 18.00 |
| NS1 | 2481 | C | T | - | 11.11 | NS4B | 6951 | C | A | - | 5.56 |
| NS1 | 2484 | T | C | - | 30.43 | NS4B | 6957 | C | T | - | 27.78 |
| NS1 | 2490 | T | C | - | 11.76 | NS4B | 6967 | G | A | Val48Ile | 10.07 |
| NS1 | 2493 | C | T | - | 10.81 | NS4B | 6979 | T | C | - | 8.81 |
| NS1 | 2511 | G | A | - | 28.74 | NS4B | 6982 | C | A | - | 9.15 |
| NS1 | 2556 | T | C | - | 5.06 | NS4B | 6990 | C | T | - | 9.43 |
| NS1 | 2577 | A | G | - | 7.89 | NS4B | 7008 | A | G | - | 34.48 |
| NS1 | 2589 | A | G | - | 27.54 | NS4B | 7014 | C | G | - | 6.67 |
| NS1 | 2619 | G | A | - | 7.04 | NS4B | 7017 | C | T | - | 6.67 |
| NS1 | 2682 | C | T | - | 14.29 | NS4B | 7018 | T | C | - | 38.10 |
| NS1 | 2685 | T | C | - | 31.71 | NS4B | 7032 | T | C | - | 10.00 |
| NS1 | 2697 | C | T | - | 15.38 | NS4B | 7038 | A | G | - | 14.58 |
| NS1 | 2700 | T | C | - | 12.82 | NS4B | 7041 | T | C | - | 34.21 |
| NS1 | 2724 | A | G | - | 12.82 | NS4B | 7059 | T | C | - | 7.64 |
| NS1 | 2731 | T | C | - | 30.56 | NS4B | 7062 | G | A | - | 19.33 |
| NS1 | 2778 | A | C | - | 13.95 | NS4B | 7083 | G | A | - | 11.26 |
| NS1 | 2781 | G | A | - | 40.43 | NS4B | 7089 | C | T | - | 10.07 |
| NS1 | 2791 | C | T | Leu124Phe | 9.38 | NS4B | 7092 | C | T | - | 10.96 |
| NS1 | 2878 | C | A | Leu153Met | 30.19 | NS4B | 7101 | C | T | - | 7.91 |
| NS1 | 2898 | T | C | - | 24.19 | NS4B | 7104 | C | T | - | 8.27 |
| NS1 | 2911 | A | T | Thr164Ser | 29.09 | NS4B | 7107 | T | C | - | 9.32 |
| NS1 | 2961 | C | T | - | 23.19 | NS4B | 7110 | C | T | - | 10.34 |
| NS1 | 2976 | A | G | - | 32.73 | NS4B | 7116 | A | G | - | 12.82 |
| NS1 | 2985 | T | A | - | 10.17 | NS4B | 7122 | C | T | - | 13.64 |
| NS1 | 2989 | A | G | Asn190Asp | 41.38 | NS4B | 7137 | T | C | - | 24.71 |
| NS1 | 2991 | C | T | - | 5.71 | NS4B | 7143 | C | T | - | 20.99 |
| NS1 | 3009 | T | C | - | 33.33 | NS4B | 7146 | C | T | - | 22.37 |
| NS1 | 3057 | G | A | Met212Ile | 10.17 | NS4B | 7155 | T | C | - | 25.00 |
| NS1 | 3060 | G | A | - | 9.84 | NS4B | 7165 | T | C | - | 13.24 |
| NS1 | 3075 | T | C | - | 38.18 | NS4B | 7186 | G | A | Val121Ile | 24.39 |
| NS1 | 3081 | A | T | - | 41.07 | NS4B | 7248 | C | T | - | 18.60 |
| NS1 | 3102 | G | A | - | 11.32 | NS4B | 7314 | A | G | - | 17.65 |
| NS1 | 3111 | C | T | - | 19.61 | NS4B | 7356 | C | T | - | 21.13 |
| NS1 | 3120 | C | T | - | 22.81 | NS4B | 7383 | T | C | - | 15.49 |
| NS1 | 3129 | A | G | - | 24.62 | NS4B | 7410 | T | C | - | 15.25 |
| NS1 | 3130 | T | C | - | 24.62 | NS4B | 7428 | C | T | - | 16.67 |

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|---|---|---|---|---|---|
| NS1 | 3156 | A | G | - | 33.33 |
| NS1 | 3165 | C | T | - | 32.14 |
| NS1 | 3168 | G | A | - | 28.13 |
| NS1 | 3174 | G | A | - | 29.41 |
| NS1 | 3186 | C | T | - | 24.69 |
| NS1 | 3201 | C | T | - | 11.69 |
| NS1 | 3204 | T | C | - | 10.53 |
| NS1 | 3213 | A | G | - | 10.67 |
| NS1 | 3222 | T | C | - | 9.72 |
| NS1 | 3234 | T | C | - | 19.28 |
| NS1 | 3313 | T | C | - | 5.96 |
| NS1 | 3324 | C | T | - | 7.58 |
| NS1 | 3327 | T | C | - | 7.52 |
| NS1 | 3330 | C | T | - | 14.60 |
| NS1 | 3339 | G | A | - | 6.29 |
| NS1 | 3357 | C | T | - | 8.00 |
| NS1 | 3366 | C | T | - | 8.70 |
| NS1 | 3373 | C | T | - | 6.14 |
| NS1 | 3402 | T | C | - | 21.59 |
| NS1 | 3432 | T | C | - | 31.03 |
| NS1 | 3438 | A | G | - | 29.82 |
| NS1 | 3447 | A | G | - | 34.78 |
| NS1 | 3474 | A | G | - | 35.48 |
| NS2A | 3489 | G | A | - | 19.23 |
| NS2A | 3526 | C | T | - | 10.00 |
| NS2A | 3552 | T | C | - | 36.36 |
| NS2A | 3597 | C | T | - | 38.46 |
| NS2A | 3604 | C | T | - | 34.00 |
| NS2A | 3612 | T | A | - | 9.26 |
| NS2A | 3624 | T | C | - | 23.53 |
| NS2A | 3629 | G | A | Arg51Lys | 21.15 |
| NS2A | 3663 | C | T | - | 25.93 |
| NS2A | 3750 | A | G | - | 4.59 |
| NS2A | 3753 | C | T | - | 15.38 |
| NS2A | 3756 | G | A | - | 2.14 |
| NS2A | 3762 | A | G | - | 3.54 |
| NS2A | 3768 | C | T | - | 16.10 |
| NS2A | 3787 | G | A | Ala104Thr | 4.62 |
| NS2A | 3803 | C | T | Ala109Val | 2.83 |
| NS2A | 3807 | C | T | - | 2.33 |
| NS2A | 3844 | C | T | - | 4.33 |
| NS2A | 3858 | A | G | - | 6.61 |
| NS2A | 3864 | G | A | - | 13.41 |
| NS2A | 3875 | C | T | Ala133Val | 12.40 |
| NS2A | 3876 | C | T | - | 8.63 |
| NS2A | 3879 | C | A | - | 9.20 |
| NS2A | 3900 | A | G | - | 5.69 |
| NS2A | 3903 | A | G | - | 5.15 |
| NS2A | 3906 | C | T | - | 4.69 |
| NS2A | 3912 | G | A | - | 7.14 |
| NS2A | 3921 | T | C | - | 10.46 |
| NS2A | 3935 | C | T | Ser153Leu | 10.06 |
| NS2A | 3936 | G | A | - | 9.39 |
| NS2A | 3942 | C | T | - | 9.50 |
| NS2A | 3958 | T | C | - | 9.38 |
| NS2A | 3960 | G | A | - | 9.28 |
| NS2A | 3966 | C | T | - | 9.25 |
| NS2A | 3975 | G | A | - | 8.72 |
| NS2A | 3981 | T | C | - | 9.57 |
| NS2A | 3991 | T | C | - | 11.04 |
| NS4B | 7518 | C | T | - | 48.65 |
| NS5 | 7752 | A | G | - | 28.57 |
| NS5 | 7776 | C | T | - | 33.33 |
| NS5 | 7777 | A | C | Met70Leu | 37.50 |
| NS5 | 7788 | G | A | - | 30.00 |
| NS5 | 7794 | G | A | - | 29.41 |
| NS5 | 7797 | G | A | - | 35.29 |
| NS5 | 7800 | G | A | - | 27.27 |
| NS5 | 7806 | T | C | - | 25.00 |
| NS5 | 7809 | T | C | - | 25.00 |
| NS5 | 7848 | A | C | - | 26.32 |
| NS5 | 7890 | A | G | - | 13.95 |
| NS5 | 7899 | C | T | - | 11.49 |
| NS5 | 7908 | C | T | - | 7.87 |
| NS5 | 7911 | C | T | - | 30.68 |
| NS5 | 7914 | C | A | - | 8.33 |
| NS5 | 7947 | G | T | - | 11.01 |
| NS5 | 7956 | G | A | - | 10.09 |
| NS5 | 7959 | C | T | - | 22.33 |
| NS5 | 7971 | C | T | - | 11.01 |
| NS5 | 7999 | T | C | - | 6.06 |
| NS5 | 8035 | A | G | Ile156Val | 10.40 |
| NS5 | 8037 | A | T | - | 10.40 |
| NS5 | 8049 | A | G | - | 10.17 |
| NS5 | 8064 | C | A | - | 9.09 |
| NS5 | 8070 | A | G | - | 11.54 |
| NS5 | 8083 | C | T | - | 13.33 |
| NS5 | 8088 | C | T | - | 11.29 |
| NS5 | 8089 | A | G | Asn174Asp | 10.66 |
| NS5 | 8115 | C | T | - | 10.87 |
| NS5 | 8121 | T | C | - | 18.89 |
| NS5 | 8184 | A | G | - | 16.67 |
| NS5 | 8241 | T | C | - | 19.30 |
| NS5 | 8284 | T | C | - | 11.86 |
| NS5 | 8306 | A | G | Lys246Arg | 15.00 |
| NS5 | 8316 | A | G | - | 21.43 |
| NS5 | 8322 | C | A | - | 22.22 |
| NS5 | 8337 | T | G | - | 33.33 |
| NS5 | 8496 | T | C | - | 15.00 |
| NS5 | 8508 | A | G | - | 20.00 |
| NS5 | 8526 | T | C | - | 16.13 |
| NS5 | 8535 | C | T | - | 21.74 |
| NS5 | 8541 | G | A | - | 28.00 |
| NS5 | 8544 | T | C | - | 27.27 |
| NS5 | 8547 | A | G | - | 17.39 |
| NS5 | 8572 | G | A | Val335Ile | 34.78 |
| NS5 | 8574 | C | T | - | 18.52 |
| NS5 | 8577 | T | C | - | 32.00 |
| NS5 | 8610 | T | C | - | 21.05 |
| NS5 | 8745 | G | A | - | 40.00 |
| NS5 | 8775 | A | G | - | 30.00 |
| NS5 | 8794 | T | C | - | 14.81 |
| NS5 | 8796 | G | A | - | 16.00 |
| NS5 | 8799 | G | T | - | 16.00 |
| NS5 | 8803 | G | A | Val412Ile | 37.50 |
| NS5 | 8823 | A | G | - | 32.00 |
| NS5 | 8829 | A | G | - | 22.22 |
| NS5 | 8832 | G | T | - | 24.00 |
| NS5 | 8859 | T | G | Ser430Arg | 22.22 |
| NS5 | 8967 | C | T | - | 26.47 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2A | 3998 | C | T | Ala174Val | 12.12 | NS5 | 8979 | C | T | - | 39.39 |
| NS2A | 4009 | T | A | Ser178Thr | 10.95 | NS5 | 9012 | A | G | - | 46.51 |
| NS2A | 4011 | T | C | - | 30.51 | NS5 | 9021 | A | G | - | 7.69 |
| NS2A | 4021 | C | T | - | 13.27 | NS5 | 9042 | T | C | - | 14.00 |
| NS2A | 4034 | A | G | Gln186Arg | 24.90 | NS5 | 9043 | C | T | - | 8.00 |
| NS2A | 4047 | T | C | - | 15.38 | NS5 | 9066 | C | T | - | 34.62 |
| NS2A | 4057 | C | T | - | 9.17 | NS5 | 9071 | G | A | Gly501Glu | 8.33 |
| NS2A | 4063 | T | C | - | 4.10 | NS5 | 9078 | C | T | - | 9.76 |
| NS2A | 4069 | A | G | Ile198Val | 8.29 | NS5 | 9079 | C | T | - | 42.50 |
| NS2A | 4071 | A | C | - | 7.96 | NS5 | 9396 | T | C | - | 25.00 |
| NS2A | 4083 | C | T | - | 25.65 | NS5 | 9399 | T | C | - | 50.00 |
| NS2A | 4098 | T | C | - | 15.08 | NS5 | 9402 | T | C | - | 45.45 |
| NS2A | 4107 | T | C | - | 14.77 | NS5 | 9416 | A | G | Glu616Gly | 25.00 |
| NS2A | 4110 | T | C | - | 6.47 | NS5 | 9501 | G | C | Gln644His | 13.56 |
| NS2A | 4113 | G | A | - | 14.63 | NS5 | 9628 | G | A | Val687Ile | 10.00 |
| NS2A | 4121 | G | A | Ser215Asn | 16.90 | NS5 | 9825 | T | C | - | 9.92 |
| NS2B | 4134 | C | T | - | 16.23 | NS5 | 9888 | G | A | - | 19.05 |
| NS2B | 4140 | G | A | - | 17.65 | NS5 | 9889 | T | C | - | 5.56 |
| NS2B | 4149 | A | G | - | 15.53 | NS5 | 9900 | T | C | - | 6.78 |
| NS2B | 4164 | C | T | - | 12.65 | NS5 | 9903 | C | T | - | 6.96 |
| NS2B | 4197 | A | G | - | 17.07 | NS5 | 9921 | G | A | - | 11.24 |
| NS2B | 4206 | T | C | - | 33.33 | NS5 | 9927 | T | C | - | 10.67 |
| NS2B | 4212 | T | C | - | 17.88 | NS5 | 9969 | G | A | - | 25.00 |
| NS2B | 4221 | T | A | - | 13.24 | NS5 | 9984 | G | A | - | 40.00 |
| NS2B | 4225 | T | C | - | 10.71 | NS5 | 9987 | G | A | - | 40.00 |
| NS2B | 4242 | C | T | - | 8.53 | NS5 | 9997 | C | A | Leu810Met | 21.05 |
| NS2B | 4248 | C | T | - | 8.94 | NS5 | 10000 | G | A | Ala811Thr | 21.05 |
| NS2B | 4251 | A | G | - | 14.96 | NS5 | 10023 | C | T | - | 9.52 |
| NS2B | 4270 | C | A | - | 7.29 | NS5 | 10059 | G | A | - | 14.81 |
| NS2B | 4278 | C | T | - | 26.58 | NS5 | 10075 | A | G | Ile836Val | 17.43 |
| NS2B | 4281 | T | C | - | 8.64 | NS5 | 10083 | C | T | - | 13.39 |
| NS2B | 4329 | A | G | - | 40.48 | NS5 | 10089 | G | A | - | 4.30 |
| NS2B | 4338 | A | G | - | 25.71 | NS5 | 10095 | G | A | - | 4.35 |
| NS2B | 4341 | C | T | - | 28.13 | NS5 | 10122 | T | A | - | 7.29 |
| NS2B | 4344 | C | T | - | 30.30 | NS5 | 10155 | T | C | - | 14.75 |
| NS2B | 4350 | T | C | - | 20.00 | NS5 | 10202 | G | A | Gly878Glu | 21.62 |
| NS2B | 4386 | G | A | - | 22.22 | NS5 | 10252 | G | A | Glu895Lys | 18.75 |
| NS2B | 4401 | G | A | - | 23.53 | 3UTR | 10389 | C | CAA | - | 2.41 |
| NS2B | 4414 | C | T | - | 22.22 | 3UTR | 10407 | T | C | - | 7.27 |

| **206** | | | | | | NS3 | 5235 | T | C | - | 18.18 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WS | | | | | | NS3 | 5304 | T | C | - | 39.53 |
| Secondary | | | | | | NS3 | 5394 | A | G | - | 40.35 |
| 5.03E+00 | | | | | | NS3 | 5409 | T | A | - | 12.50 |
| 5 | | | | | | NS3 | 5502 | G | A | - | 8.51 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5559 | A | T | - | 7.69 |
| C | 231 | A | G | - | 12.35 | NS3 | 5649 | G | A | - | 35.56 |
| C | 327 | T | C | - | 9.09 | NS3 | 5667 | G | A | - | 25.53 |
| C | 411 | T | C | - | 37.14 | NS3 | 5730 | T | C | - | 32.76 |
| prM/M | 450 | C | T | - | 36.67 | NS3 | 5751 | T | C | - | 15.09 |
| prM/M | 606 | C | T | - | 12.90 | NS3 | 5754 | A | G | - | 15.69 |
| prM/M | 651 | A | G | - | 19.61 | NS3 | 5835 | A | G | - | 22.92 |
| prM/M | 717 | G | A | - | 17.65 | NS3 | 5844 | G | A | - | 12.77 |
| prM/M | 798 | C | T | - | 19.77 | NS3 | 6033 | C | T | - | 10.26 |
| prM/M | 820 | T | C | - | 34.83 | NS3 | 6157 | T | C | - | 15.00 |
| prM/M | 855 | C | T | - | 8.43 | NS3 | 6167 | A | G | Lys549Arg | 11.63 |
| prM/M | 885 | C | T | - | 9.30 | NS3 | 6324 | T | C | - | 20.29 |
| E | 954 | A | C | - | 18.89 | NS3 | 6330 | C | T | - | 25.35 |
| E | 1008 | T | C | - | 15.13 | NS4A | 6423 | C | T | - | 5.88 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 1023 | T | C | - | 10.47 | NS4A | 6471 | T | C | - | 23.68 |
| E | 1040 | C | CA | Asn37fs | 8.33 | NS4A | 6512 | G | A | Ser46Asn | 6.67 |
| E | 1287 | T | C | - | 19.70 | NS4A | 6528 | T | C | - | 44.44 |
| E | 1302 | A | G | - | 43.86 | NS4A | 6585 | A | G | - | 26.67 |
| E | 1353 | C | T | - | 5.33 | NS4A | 6606 | T | C | - | 20.00 |
| E | 1368 | C | T | - | 14.06 | NS4A | 6652 | A | G | Ile93Val | 35.21 |
| E | 1479 | C | T | - | 30.43 | NS4A | 6711 | G | GT | Leu115fs | 6.15 |
| E | 1545 | C | T | - | 5.62 | NS4B | 6864 | C | T | - | 12.05 |
| E | 1689 | T | C | - | 9.09 | NS4B | 6871 | G | A | Gly16Arg | 12.36 |
| E | 1701 | T | C | - | 30.36 | NS4B | 6951 | C | A | - | 13.82 |
| E | 1710 | G | T | - | 9.84 | NS4B | 6966 | T | C | - | 23.77 |
| E | 1959 | T | C | - | 10.71 | NS4B | 6984 | A | G | - | 26.24 |
| E | 1972 | C | T | His346Tyr | 8.62 | NS4B | 7008 | G | A | - | 8.16 |
| E | 1986 | C | T | - | 46.58 | NS4B | 7038 | G | A | - | 41.27 |
| E | 2223 | T | C | - | 24.14 | NS4B | 7059 | T | C | - | 21.93 |
| E | 2244 | C | T | - | 40.00 | NS4B | 7137 | T | C | - | 28.57 |
| E | 2256 | C | T | - | 13.19 | NS4B | 7161 | T | C | - | 15.09 |
| E | 2276 | C | T | Ala447Val | 40.00 | NS4B | 7449 | T | C | - | 23.44 |
| E | 2319 | C | A | - | 30.97 | NS5 | 7656 | G | A | - | 31.71 |
| E | 2328 | A | T | - | 12.93 | NS5 | 7719 | G | A | - | 12.50 |
| E | 2397 | G | A | - | 33.33 | NS5 | 7758 | G | A | - | 27.59 |
| NS1 | 2685 | T | C | - | 12.77 | NS5 | 7767 | T | C | - | 33.33 |
| NS1 | 2791 | C | T | Leu124Phe | 20.31 | NS5 | 7999 | T | C | - | 46.36 |
| NS1 | 2911 | A | T | Thr164Ser | 14.04 | NS5 | 8103 | T | C | - | 8.86 |
| NS1 | 2991 | C | T | - | 17.05 | NS5 | 8139 | C | T | - | 11.76 |
| NS1 | 3129 | A | G | - | 10.87 | NS5 | 8298 | C | T | - | 23.21 |
| NS1 | 3330 | C | T | - | 22.66 | NS5 | 8562 | T | C | - | 32.61 |
| NS2A | 3489 | G | A | - | 35.56 | NS5 | 8574 | C | T | - | 8.16 |
| NS2A | 3595 | T | C | Phe40Leu | 21.43 | NS5 | 8604 | C | T | - | 28.57 |
| NS2A | 3612 | T | A | - | 16.67 | NS5 | 8640 | G | A | - | 33.33 |
| NS2A | 3743 | C | T | Ala89Val | 11.51 | NS5 | 8658 | T | C | - | 46.15 |
| NS2A | 3750 | A | G | - | 11.49 | NS5 | 8760 | A | G | - | 20.00 |
| NS2A | 3801 | C | T | - | 13.21 | NS5 | 8823 | A | G | - | 17.39 |
| NS2A | 3844 | C | T | - | 8.96 | NS5 | 9399 | C | T | - | 26.67 |
| NS2A | 3858 | A | G | - | 17.26 | NS5 | 9424 | T | C | - | 45.83 |
| NS2A | 3876 | C | T | - | 6.28 | NS5 | 9491 | T | C | Ile641Thr | 11.11 |
| NS2A | 3906 | C | T | - | 12.72 | NS5 | 9576 | T | C | - | 23.29 |
| NS2A | 3910 | T | C | - | 11.50 | NS5 | 9623 | GA | G | Lys686fs | 4.00 |
| NS2A | 4034 | A | G | Gln186Arg | 22.15 | NS5 | 9660 | A | T | - | 25.00 |
| NS2A | 4063 | T | C | - | 13.25 | NS5 | 9725 | A | T | Lys719Ile | 22.73 |
| NS2A | 4110 | T | C | - | 18.25 | NS5 | 9768 | A | G | - | 23.81 |
| NS2B | 4206 | T | C | - | 36.07 | NS5 | 9789 | T | C | - | 31.11 |
| NS2B | 4249 | G | A | Val40Ile | 7.75 | NS5 | 9795 | G | A | - | 25.56 |
| NS2B | 4251 | A | G | - | 10.40 | NS5 | 9810 | T | C | - | 14.15 |
| NS2B | 4257 | C | T | - | 8.18 | NS5 | 9840 | T | C | - | 19.42 |
| NS2B | 4350 | C | T | - | 29.09 | NS5 | 9849 | A | G | - | 20.34 |
| NS2B | 4414 | T | C | - | 26.32 | NS5 | 9942 | T | C | - | 28.57 |
| NS2B | 4482 | G | A | - | 28.57 | NS5 | 10071 | A | T | Glu834Asp | 11.11 |
| NS3 | 4587 | C | T | - | 15.00 | NS5 | 10089 | G | A | - | 7.50 |
| NS3 | 4854 | G | A | - | 21.69 | 3UTR | 10389 | T | TAA | - | 44.26 |
| NS3 | 5112 | A | G | - | 15.38 | 3UTR | 10407 | C | T | - | 26.56 |

| **207** | | | | | | NS3 | 4854 | A | G | - | 41.51 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| WS | | | | | | NS3 | 4875 | C | T | - | 48.21 |
| Secondary | | | | | | NS3 | 4905 | G | A | - | 42.55 |
| 1.08E+01 | | | | | | NS3 | 4944 | C | T | - | 31.71 |
| 5 | | | | | | NS3 | 4959 | T | C | - | 41.03 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 4974 | T | C | - | 13.16 |
| C | 201 | T | C | - | 26.19 | NS3 | 5026 | G | GA | Ser171fs | 4.17 |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 219 | C | T | - | 8.11 | NS3 | 5070 | C | T | - | 37.50 |
| C | 315 | G | A | - | 18.18 | NS3 | 5190 | C | T | - | 20.69 |
| C | 327 | C | T | - | 33.33 | NS3 | 5235 | T | C | - | 34.15 |
| C | 384 | C | T | - | 36.84 | NS3 | 5277 | C | T | - | 37.50 |
| C | 387 | G | A | - | 40.00 | NS3 | 5283 | A | G | - | 42.31 |
| prM/M | 445 | T | C | - | 35.71 | NS3 | 5304 | T | C | - | 39.29 |
| prM/M | 450 | C | T | - | 19.30 | NS3 | 5572 | G | A | Val351Ile | 27.59 |
| prM/M | 508 | C | T | - | 37.21 | NS3 | 5613 | T | C | - | 38.10 |
| prM/M | 567 | G | A | - | 40.91 | NS3 | 5649 | G | A | - | 21.05 |
| prM/M | 606 | C | T | - | 25.81 | NS3 | 5667 | G | A | - | 31.58 |
| prM/M | 651 | G | A | - | 42.11 | NS3 | 5730 | C | T | - | 22.73 |
| prM/M | 717 | G | A | - | 33.33 | NS3 | 5796 | T | C | - | 18.18 |
| prM/M | 852 | A | G | - | 50.00 | NS3 | 5829 | T | C | - | 18.18 |
| prM/M | 852 | G | A | - | 50.00 | NS3 | 5859 | T | C | - | 25.00 |
| prM/M | 864 | C | T | - | 33.33 | NS3 | 5979 | A | C | - | 27.78 |
| prM/M | 873 | A | C | - | 30.95 | NS3 | 6009 | T | C | - | 36.36 |
| prM/M | 893 | C | T | Ala152Val | 10.00 | NS3 | 6033 | C | T | - | 15.38 |
| prM/M | 894 | C | A | - | 37.50 | NS3 | 6069 | G | T | - | 20.69 |
| prM/M | 921 | C | T | - | 30.00 | NS3 | 6157 | T | C | - | 23.81 |
| E | 1005 | C | A | - | 42.55 | NS3 | 6159 | G | A | - | 19.05 |
| E | 1023 | T | C | - | 8.00 | NS3 | 6198 | C | T | - | 17.65 |
| E | 1102 | C | T | - | 42.11 | NS3 | 6330 | T | C | - | 35.00 |
| E | 1287 | T | C | - | 13.79 | NS4A | 6378 | T | C | - | 24.07 |
| E | 1293 | T | C | - | 48.28 | NS4A | 6379 | T | C | - | 24.53 |
| E | 1353 | C | T | - | 17.65 | NS4A | 6462 | T | C | - | 37.50 |
| E | 1413 | C | A | - | 17.39 | NS4A | 6471 | T | C | - | 36.00 |
| E | 1473 | C | T | - | 30.43 | NS4A | 6491 | A | G | Lys39Arg | 38.30 |
| E | 1590 | G | A | - | 42.37 | NS4A | 6496 | T | C | Tyr41His | 33.33 |
| E | 1641 | G | A | - | 28.57 | NS4A | 6500 | C | A | Thr42Asn | 30.51 |
| E | 1842 | T | C | - | 25.00 | NS4A | 6528 | T | C | - | 35.85 |
| E | 1860 | C | T | - | 27.78 | NS4A | 6547 | C | T | - | 32.69 |
| E | 1863 | G | A | - | 47.37 | NS4A | 6556 | C | T | - | 33.90 |
| E | 1995 | A | G | - | 42.86 | NS4A | 6562 | G | A | Ala63Thr | 31.58 |
| E | 2055 | C | T | - | 31.03 | NS4A | 6564 | G | A | - | 38.60 |
| E | 2073 | C | T | - | 48.00 | NS4A | 6585 | G | A | - | 34.92 |
| E | 2223 | T | C | - | 27.12 | NS4A | 6633 | T | C | - | 20.59 |
| E | 2247 | T | C | - | 24.14 | NS4A | 6681 | A | G | - | 28.57 |
| E | 2256 | C | T | - | 10.34 | NS4A | 6732 | C | T | - | 8.89 |
| E | 2276 | C | T | Ala447Val | 10.29 | NS4B | 6841 | C | T | - | 11.63 |
| E | 2289 | C | T | - | 24.29 | NS4B | 6864 | T | C | - | 26.09 |
| E | 2307 | C | T | - | 32.43 | NS4B | 6871 | A | G | Arg16Gly | 22.45 |
| E | 2319 | C | A | - | 10.53 | NS4B | 6879 | T | C | - | 17.02 |
| E | 2328 | A | T | - | 43.28 | NS4B | 6948 | T | C | - | 38.75 |
| NS1 | 2448 | A | G | - | 34.00 | NS4B | 6957 | T | C | - | 6.17 |
| NS1 | 2484 | T | C | - | 29.82 | NS4B | 7008 | G | A | - | 49.04 |
| NS1 | 2589 | A | G | - | 41.03 | NS4B | 7018 | C | T | - | 5.66 |
| NS1 | 2781 | G | A | - | 48.89 | NS4B | 7029 | T | A | - | 44.00 |
| NS1 | 2878 | C | A | Leu153Met | 24.14 | NS4B | 7038 | A | G | - | 17.35 |
| NS1 | 2898 | T | C | - | 25.93 | NS4B | 7041 | C | T | - | 47.47 |
| NS1 | 2952 | A | G | - | 39.29 | NS4B | 7059 | T | C | - | 26.83 |
| NS1 | 2961 | C | T | - | 43.48 | NS4B | 7062 | G | A | - | 48.78 |
| NS1 | 2976 | A | G | - | 30.43 | NS4B | 7134 | C | T | - | 20.51 |
| NS1 | 2991 | T | C | - | 21.05 | NS4B | 7137 | T | C | - | 40.00 |
| NS1 | 3075 | T | C | - | 20.59 | NS4B | 7165 | T | C | - | 29.63 |
| NS1 | 3114 | A | C | - | 43.75 | NS4B | 7186 | A | G | ile121Val | 17.14 |
| NS1 | 3152 | C | CA | Asn246fs | 5.56 | NS4B | 7314 | A | G | - | 33.33 |
| NS1 | 3234 | T | C | - | 29.09 | NS4B | 7383 | T | C | - | 45.24 |
| NS1 | 3330 | C | T | - | 14.52 | NS4B | 7518 | C | T | - | 40.63 |
| NS1 | 3342 | C | T | - | 5.80 | NS5 | 7758 | A | G | - | 30.00 |
| NS1 | 3402 | T | C | - | 43.48 | NS5 | 7812 | T | C | - | 28.57 |

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|---|---|---|---|---|---|
| NS2A | 3489 | G | A | - | 29.17 |
| NS2A | 3624 | T | C | - | 33.33 |
| NS2A | 3629 | G | A | Arg51Lys | 36.00 |
| NS2A | 3753 | C | T | - | 40.54 |
| NS2A | 3801 | C | T | - | 18.97 |
| NS2A | 3864 | G | A | - | 37.61 |
| NS2A | 3875 | C | T | Ala133Val | 30.11 |
| NS2A | 3876 | T | C | - | 37.89 |
| NS2A | 3879 | C | A | - | 26.21 |
| NS2A | 3910 | T | C | - | 13.85 |
| NS2A | 3921 | T | C | - | 38.06 |
| NS2A | 4011 | T | C | - | 41.58 |
| NS2A | 4034 | A | G | Gln186Arg | 19.28 |
| NS2A | 4063 | C | T | - | 43.75 |
| NS2A | 4083 | C | T | - | 38.03 |
| NS2A | 4107 | T | C | - | 7.89 |
| NS2A | 4110 | T | C | - | 21.79 |
| NS2A | 4121 | G | A | Ser215Asn | 8.57 |
| NS2B | 4149 | A | G | - | 8.57 |
| NS2B | 4164 | C | T | - | 40.32 |
| NS2B | 4206 | T | C | - | 32.84 |
| NS2B | 4249 | G | A | Val40Ile | 12.07 |
| NS2B | 4278 | C | T | - | 48.78 |
| NS2B | 4329 | A | G | - | 46.15 |
| NS2B | 4338 | A | G | - | 36.36 |
| NS2B | 4341 | C | T | - | 27.27 |
| NS2B | 4344 | C | T | - | 34.78 |
| NS2B | 4350 | T | C | - | 45.45 |
| NS3 | 4806 | T | C | - | 34.38 |
| NS3 | 4812 | C | T | - | 26.47 |
| NS3 | 4830 | A | G | - | 32.35 |
| NS5 | 7911 | C | T | - | 33.33 |
| NS5 | 7959 | C | T | - | 46.30 |
| NS5 | 7999 | T | C | - | 29.17 |
| NS5 | 8103 | T | C | - | 20.93 |
| NS5 | 8127 | T | C | - | 24.44 |
| NS5 | 8184 | A | G | - | 33.33 |
| NS5 | 8241 | T | C | - | 20.51 |
| NS5 | 8284 | T | C | - | 20.00 |
| NS5 | 8310 | T | C | - | 15.38 |
| NS5 | 8544 | C | T | - | 41.67 |
| NS5 | 8569 | G | A | Val334Ile | 24.24 |
| NS5 | 8574 | C | T | - | 44.12 |
| NS5 | 8604 | C | T | - | 26.32 |
| NS5 | 8803 | G | A | Val412Ile | 31.25 |
| NS5 | 8967 | C | T | - | 25.00 |
| NS5 | 9018 | C | T | - | 13.04 |
| NS5 | 9066 | C | T | - | 33.33 |
| NS5 | 9079 | C | T | - | 38.46 |
| NS5 | 9501 | G | C | Gln644His | 37.93 |
| NS5 | 9628 | G | A | Val687Ile | 34.00 |
| NS5 | 9795 | G | A | - | 32.65 |
| NS5 | 9810 | T | C | - | 17.02 |
| NS5 | 9840 | T | C | - | 23.81 |
| NS5 | 9849 | A | G | - | 37.84 |
| NS5 | 9888 | A | G | - | 8.96 |
| NS5 | 9942 | T | C | - | 27.03 |
| NS5 | 10083 | C | T | - | 39.22 |
| 3UTR | 10389 | C | CA | - | 19.51 |
| 3UTR | 10407 | T | C | - | 31.91 |
| 3UTR | 10443 | T | TA | - | 3.92 |
| 3UTR | 10611 | C | CAA | - | 36.36 |

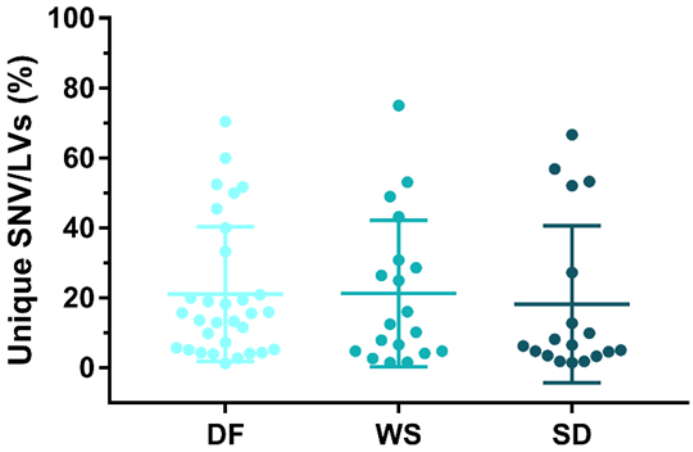
| **208** | | | | | |
|---|---|---|---|---|---|
| SD | | | | | |
| Primary | | | | | |
| 2.86E+00 | | | | | |
| 24 | | | | | |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % |
| C | 411 | T | C | - | 35.71 |
| prM/M | 450 | C | T | - | 32.20 |
| prM/M | 523 | G | A | Asp29Asn | 9.09 |
| prM/M | 606 | C | T | - | 29.09 |
| prM/M | 820 | T | C | - | 30.99 |
| prM/M | 864 | T | C | - | 12.20 |
| prM/M | 912 | G | A | - | 15.19 |
| E | 954 | A | C | - | 19.67 |
| E | 1023 | T | C | - | 6.94 |
| E | 1040 | C | CA | Asn37fs | 7.41 |
| E | 1302 | A | G | - | 25.00 |
| E | 1353 | C | T | - | 28.57 |
| E | 1479 | C | T | - | 15.38 |
| E | 1701 | T | C | - | 36.84 |
| E | 1860 | C | T | - | 31.25 |
| E | 1955 | C | T | Thr340Ile | 10.00 |
| E | 1959 | T | C | - | 19.35 |
| E | 1986 | C | T | - | 10.34 |
| E | 2055 | C | T | - | 21.15 |
| E | 2082 | A | G | - | 27.91 |
| E | 2169 | A | G | - | 11.43 |
| NS3 | 5304 | T | C | - | 45.00 |
| NS3 | 5394 | A | G | - | 27.50 |
| NS3 | 5478 | G | A | - | 25.93 |
| NS3 | 5502 | G | A | - | 20.00 |
| NS3 | 5730 | C | T | - | 40.00 |
| NS3 | 5766 | C | T | - | 9.09 |
| NS3 | 5796 | T | C | - | 11.63 |
| NS3 | 5835 | A | G | - | 21.21 |
| NS3 | 5844 | G | A | - | 42.42 |
| NS3 | 5979 | A | C | - | 25.00 |
| NS3 | 6157 | T | C | - | 25.93 |
| NS3 | 6279 | G | A | - | 7.50 |
| NS3 | 6324 | T | C | - | 33.90 |
| NS4A | 6471 | T | C | - | 7.04 |
| NS4A | 6585 | A | G | - | 9.23 |
| NS4A | 6606 | T | C | - | 19.18 |
| NS4A | 6652 | A | G | Ile93Val | 19.57 |
| NS4A | 6711 | G | GT | Leu115fs | 6.98 |
| NS4B | 6871 | G | A | Gly16Arg | 5.56 |
| NS4B | 6943 | C | T | - | 19.05 |
| NS4B | 6957 | T | C | - | 8.25 |
| NS4B | 6966 | T | C | - | 12.63 |
| NS4B | 6984 | A | G | - | 18.92 |
| NS4B | 7038 | A | G | - | 46.88 |
| NS4B | 7059 | T | C | - | 42.86 |
| NS4B | 7083 | G | A | - | 14.29 |
| NS4B | 7092 | C | T | - | 13.33 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 2220 | G | A | - | 20.83 | NS4B | 7137 | T | C | - | 24.14 |
| E | 2223 | C | T | - | 29.17 | NS4B | 7449 | T | C | - | 28.85 |
| E | 2244 | C | T | - | 20.69 | NS4B | 7542 | T | C | - | 36.84 |
| E | 2256 | C | T | - | 28.57 | NS5 | 7599 | A | G | - | 16.67 |
| E | 2276 | C | T | Ala447Val | 27.87 | NS5 | 7656 | G | A | - | 38.46 |
| E | 2318 | T | C | Val461Ala | 21.67 | NS5 | 7658 | A | G | Lys30Arg | 30.77 |
| E | 2319 | C | A | - | 37.29 | NS5 | 7686 | C | T | - | 23.81 |
| E | 2397 | G | A | - | 23.29 | NS5 | 7999 | C | T | - | 39.39 |
| NS1 | 2448 | A | G | - | 33.33 | NS5 | 8211 | C | T | - | 17.24 |
| NS1 | 3162 | T | A | Phe247Leu | 25.71 | NS5 | 8298 | C | T | - | 20.51 |
| NS1 | 3330 | C | T | - | 32.00 | NS5 | 8562 | T | C | - | 41.18 |
| NS1 | 3342 | C | T | - | 11.93 | NS5 | 8569 | G | A | Val334Ile | 19.61 |
| NS2A | 3489 | G | A | - | 20.00 | NS5 | 8604 | C | T | - | 22.86 |
| NS2A | 3801 | C | T | - | 22.76 | NS5 | 8640 | G | A | - | 33.33 |
| NS2A | 3910 | T | C | - | 20.10 | NS5 | 8859 | G | T | Arg430Ser | 10.53 |
| NS2A | 4034 | A | G | Gln186Arg | 42.25 | NS5 | 9399 | T | C | - | 28.57 |
| NS2A | 4069 | A | G | Ile198Val | 7.44 | NS5 | 9477 | C | T | - | 21.62 |
| NS2A | 4110 | T | C | - | 21.74 | NS5 | 9576 | T | C | - | 23.40 |
| NS2B | 4149 | A | G | - | 5.56 | NS5 | 9725 | A | T | Lys719Ile | 17.74 |
| NS2B | 4206 | T | C | - | 39.34 | NS5 | 9768 | A | G | - | 23.60 |
| NS2B | 4249 | G | A | Val40Ile | 25.51 | NS5 | 9789 | T | C | - | 15.87 |
| NS2B | 4257 | C | T | - | 24.21 | NS5 | 9795 | G | A | - | 45.90 |
| NS2B | 4350 | C | T | - | 11.11 | NS5 | 9942 | T | C | - | 33.33 |
| NS2B | 4482 | G | A | - | 32.00 | 3UTR | 10386 | T | TCA | - | 17.31 |
| NS3 | 4562 | G | A | Gly14Glu | 12.90 | 3UTR | 10387 | C | CAT | - | 17.31 |
| NS3 | 4707 | G | A | - | 32.14 | 3UTR | 10388 | A | AC | - | 32.00 |
| NS3 | 4974 | C | T | - | 50.00 | 3UTR | 10389 | A | C | - | 26.00 |
| NS3 | 5295 | C | T | - | 8.57 | 3UTR | 10407 | C | T | - | 25.00 |

| **209** | | | | | | NS3 | 4662 | T | C | - | 15.38 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SD | | | | | | NS3 | 4806 | C | T | - | 28.57 |
| Primary | | | | | | NS3 | 4854 | G | A | - | 18.37 |
| 7.52E+00 | | | | | | NS3 | 4974 | T | C | - | 46.30 |
| 6 | | | | | | NS3 | 5026 | G | A | Glu169Lys | 7.41 |
| Genomic region | POS | REF | ALT | Aa substitution | Freq % | NS3 | 5235 | T | C | - | 17.78 |
| C | 168 | A | G | - | 8.11 | NS3 | 5544 | G | A | - | 12.50 |
| C | 181 | C | T | - | 12.82 | NS3 | 5550 | A | G | - | 12.50 |
| C | 201 | T | C | - | 47.83 | NS3 | 5559 | A | T | - | 17.65 |
| C | 213 | A | G | - | 8.51 | NS3 | 5613 | T | C | - | 48.00 |
| C | 327 | T | C | - | 22.22 | NS3 | 5631 | C | T | - | 18.18 |
| C | 336 | T | C | - | 13.33 | NS3 | 5649 | G | A | - | 16.00 |
| C | 384 | C | T | - | 47.06 | NS3 | 5667 | G | A | - | 17.39 |
| C | 387 | G | A | - | 44.44 | NS3 | 5704 | G | A | Val395Ile | 9.09 |
| prM/M | 508 | C | T | - | 42.31 | NS3 | 5751 | T | C | - | 12.12 |
| prM/M | 531 | T | C | - | 8.06 | NS3 | 5754 | A | G | - | 11.43 |
| prM/M | 606 | C | T | - | 15.91 | NS3 | 5910 | A | G | - | 11.76 |
| prM/M | 723 | C | T | - | 38.46 | NS3 | 6069 | G | T | - | 40.00 |
| prM/M | 876 | G | A | - | 7.69 | NS3 | 6108 | A | G | - | 13.79 |
| prM/M | 885 | C | T | - | 7.58 | NS3 | 6157 | T | C | - | 16.67 |
| prM/M | 893 | C | T | Ala152Val | 12.31 | NS3 | 6177 | T | C | - | 11.11 |
| prM/M | 912 | G | A | - | 12.12 | NS3 | 6186 | C | T | - | 9.09 |
| E | 960 | T | C | - | 7.79 | NS3 | 6279 | G | A | - | 12.50 |
| E | 1023 | T | C | - | 12.50 | NS3 | 6300 | C | T | - | 47.06 |
| E | 1094 | C | T | Pro53Leu | 15.63 | NS3 | 6330 | C | T | - | 30.77 |
| E | 1599 | A | G | - | 16.33 | NS3 | 6333 | A | G | - | 9.52 |
| E | 1689 | T | C | - | 20.59 | NS3 | 6339 | A | G | - | 7.55 |
| E | 1858 | A | G | Ile308Val | 13.79 | NS3 | 6342 | A | C | - | 7.41 |
| E | 1860 | C | T | - | 42.86 | NS4A | 6378 | T | C | - | 44.90 |
| E | 2007 | C | T | - | 16.67 | NS4A | 6379 | T | C | - | 44.90 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| E | 2220 | G | A | - | 34.62 | NS4A | 6471 | T | C | - | 27.78 |
| E | 2223 | C | T | - | 30.77 | NS4A | 6538 | C | T | - | 6.90 |
| E | 2328 | A | T | - | 8.75 | NS4A | 6561 | A | T | - | 7.81 |
| E | 2367 | G | A | - | 6.25 | NS4A | 6585 | A | G | - | 32.86 |
| E | 2395 | C | T | - | 7.46 | NS4A | 6588 | C | T | - | 8.57 |
| E | 2401 | C | T | - | 6.94 | NS4A | 6615 | G | A | - | 10.00 |
| NS1 | 2448 | G | A | - | 30.77 | NS4A | 6645 | A | G | - | 12.50 |
| NS1 | 2494 | G | A | Val25Met | 29.23 | NS4A | 6648 | T | C | - | 8.62 |
| NS1 | 2808 | C | T | - | 7.69 | NS4A | 6651 | C | T | - | 8.93 |
| NS1 | 2910 | T | C | - | 8.89 | NS4A | 6666 | T | C | - | 11.36 |
| NS1 | 2911 | A | T | Thr164Ser | 20.45 | NS4A | 6678 | A | G | - | 9.76 |
| NS1 | 2952 | A | G | - | 13.04 | NS4A | 6681 | A | G | - | 47.22 |
| NS1 | 2961 | C | T | - | 45.45 | NS4A | 6727 | T | C | - | 9.09 |
| NS1 | 2976 | A | G | - | 48.72 | NS4B | 6864 | C | T | - | 21.82 |
| NS1 | 2991 | C | T | - | 31.91 | NS4B | 6870 | T | G | Phe15Leu | 12.50 |
| NS1 | 3075 | T | C | - | 48.78 | NS4B | 6871 | G | A | Gly16Arg | 19.30 |
| NS1 | 3114 | C | A | - | 39.39 | NS4B | 6879 | T | C | - | 47.46 |
| NS1 | 3129 | A | G | - | 31.43 | NS4B | 6880 | A | G | Thr19Ala | 10.94 |
| NS1 | 3152 | C | CA | Asn246fs | 5.41 | NS4B | 6884 | C | T | Thr20Ile | 15.63 |
| NS2A | 3489 | G | A | - | 14.71 | NS4B | 6943 | C | T | - | 24.69 |
| NS2A | 3593 | CT | C | Phe40fs | 4.69 | NS4B | 6951 | C | A | - | 9.59 |
| NS2A | 3612 | T | A | - | 11.94 | NS4B | 6957 | T | C | - | 10.67 |
| NS2A | 3750 | A | G | - | 12.24 | NS4B | 7008 | G | A | - | 7.34 |
| NS2A | 3753 | C | T | - | 50.00 | NS4B | 7014 | C | G | - | 7.41 |
| NS2A | 3840 | A | T | - | 5.38 | NS4B | 7038 | A | G | - | 10.99 |
| NS2A | 3858 | A | G | - | 13.53 | NS4B | 7083 | G | A | - | 21.79 |
| NS2A | 3874 | G | A | Val133Ile | 7.30 | NS4B | 7092 | C | T | - | 19.74 |
| NS2A | 3876 | C | T | - | 33.08 | NS4B | 7161 | T | C | - | 12.12 |
| NS2A | 3879 | C | A | - | 49.62 | NS4B | 7248 | C | T | - | 38.89 |
| NS2A | 3991 | T | C | - | 4.14 | NS4B | 7449 | T | C | - | 8.70 |
| NS2A | 3998 | C | T | Ala174Val | 4.48 | NS4B | 7551 | C | T | - | 31.58 |
| NS2A | 4009 | T | A | Ser178Thr | 8.09 | NS5 | 7656 | G | A | - | 17.24 |
| NS2A | 4021 | C | T | - | 7.63 | NS5 | 7947 | G | T | - | 7.58 |
| NS2A | 4047 | T | C | - | 7.53 | NS5 | 7996 | T | C | - | 22.67 |
| NS2A | 4063 | T | C | - | 25.26 | NS5 | 7999 | T | C | - | 9.09 |
| NS2A | 4069 | A | G | Ile198Val | 20.59 | NS5 | 8089 | A | G | Asn174Asp | 16.39 |
| NS2A | 4110 | C | T | - | 39.42 | NS5 | 8103 | T | C | - | 17.65 |
| NS2A | 4121 | G | A | Ser215Asn | 6.67 | NS5 | 8235 | T | C | - | 19.51 |
| NS2B | 4134 | C | T | - | 6.90 | NS5 | 8574 | C | T | - | 12.12 |
| NS2B | 4140 | G | A | - | 7.59 | NS5 | 8604 | C | T | - | 26.32 |
| NS2B | 4305 | T | C | - | 10.64 | NS5 | 8610 | T | C | - | 16.67 |
| NS2B | 4310 | A | G | Lys60Arg | 10.64 | NS5 | 8823 | A | G | - | 18.18 |
| NS2B | 4323 | G | A | - | 10.00 | NS5 | 9399 | C | T | - | 14.29 |
| NS2B | 4341 | C | T | - | 10.34 | NS5 | 9477 | C | T | - | 44.44 |
| NS2B | 4350 | C | T | - | 34.29 | NS5 | 9628 | G | A | Val687Ile | 20.00 |
| NS2B | 4414 | C | T | - | 31.58 | NS5 | 9810 | T | C | - | 23.26 |
| NS3 | 4566 | A | G | - | 18.52 | NS5 | 9840 | T | C | - | 13.58 |
| NS3 | 4602 | A | G | - | 17.14 | NS5 | 9849 | A | G | - | 16.09 |
| NS3 | 4604 | G | A | Arg28Lys | 10.81 | NS5 | 9888 | A | G | - | 9.72 |
| NS3 | 4620 | T | C | - | 17.14 | NS5 | 10075 | G | A | Val836Ile | 9.23 |
| NS3 | 4629 | T | C | - | 21.43 | NS5 | 10089 | G | A | - | 23.08 |
| NS3 | 4635 | C | T | - | 22.22 | 3UTR | 10389 | CA | C | - | 38.60 |
| NS3 | 4641 | T | C | - | 17.86 | 3UTR | 10407 | C | T | - | 31.91 |

**Figure S4**—Percentage of unique variants in mutant swarms of cases grouped by clinical classification. Each point represents the value of a single sample, and error bars represent the mean with standard deviation for each category.




ANOVA test for means' comparison, $p>0.05$. DF: dengue fever; WS: dengue with warning signs; SD: severe dengue.

**Table S5**—Repetitive NS-iSNVs + UTRiSNVs clustered by subgroups according to their interhost frequency within the clinical categories. In bold: interhost frequencies higher than 25%. Aa: amino acid; DF: dengue fever; WS: dengue with warning signs; SD: severe dengue; P: primary infection; S: secondary infection; freq: frequency.

| Genome region | iSNV | Aa substitution | Relevant aa subs. | Total (n) | DF (%) | WS (%) | SD (%) | Intrahost freq range DF | Intrahost freq range WS | Intrahost freq range SD | P (%) | S (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subgroup 1 - DF (16-48%, n=5-15) | | | | | | | | | | | | |
| E | G1595T | Trp220Leu | Yes | 15 | 48 | 0 | 0 | 1-2 | - | - | 28 | 14 |
| E | T2276C | Val447Ala | - | 5 | 16 | 0 | 0 | **1-34** | - | - | 10 | 3 |
| E | A2303G | Lys456Arg | - | 7 | 23 | 0 | 0 | 1-2 | - | - | 10 | 10 |
| E | C2308T | Leu458Phe | Yes | 14 | 45 | 0 | 0 | 1-2 | - | - | 28 | 10 |
| E | G2315T | Gly460Val | - | 9 | 29 | 0 | 0 | 1-2 | - | - | 15 | 10 |
| NS2A | C3716T | Ala80Val | - | 10 | 32 | 0 | 0 | 1 | - | - | 21 | 7 |
| NS3 | A5543C | Glu341Ala | Yes | 7 | 23 | 0 | 0 | 1-2 | - | - | 13 | 7 |
| NS3 | C5839T | Arg440Trp | Yes | 13 | 42 | 0 | 0 | 1-3 | - | - | 26 | 10 |
| NS3 | G6220A | Val567Ile | - | 6 | 19 | 0 | 0 | 1-17 | - | - | 8 | 10 |
| NS3 | C6365T | Ala615Val | - | 13 | 42 | 0 | 0 | 1-2 | - | - | 28 | 7 |
| NS4A | A6418G | Thr15Ala | Yes | 5 | 16 | 0 | 0 | 1-2 | - | - | 13 | 0 |
| NS4A | C6655T | Leu94Phe | Yes | 6 | 19 | 0 | 0 | 1 | - | - | 8 | 10 |
| NS4B | A6913G | Ile30Val | - | 5 | 16 | 0 | 0 | 1 | - | - | 13 | 0 |
| NS5 | T7763C | Phe65Ser | Yes | 5 | 16 | 0 | 0 | 3-7 | - | - | 8 | 7 |
| NS5 | G8184C | Leu205Phe | Yes | 6 | 19 | 0 | 0 | 1-2 | - | - | 13 | 3 |
| NS5 | G8602A | Asp345Asn | Yes | 12 | 39 | 0 | 0 | 1-4 | - | - | 21 | 14 |
| NS5 | C9877T | Arg770Cys | Yes | 5 | 16 | 0 | 0 | 1 | - | - | 8 | 7 |
| 3UTR | 10389delAA | - | - | 5 | 16 | 0 | 0 | **4-26** | - | - | 8 | 7 |
| 3UTR | 10613insA | - | - | 5 | 16 | 0 | 0 | 1-6 | - | - | 8 | 7 |

| Subgroup 2 - WS+SD (5-26%, n=1-5 WS; 6-50%, n=1-9 SD) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| prM/M | C893T | Ala152Val | - | 9 | 0 | 16 | 33 | - | **3-15** | **4-32** | 13 | 14 |
| E | A1001C | Asp22Ala | Yes | 6 | 0 | 26 | 6 | - | 1 | 1 | 10 | 7 |
| E | A2023G | Ser363Ala | Yes | 8 | 0 | 26 | 17 | - | **1-38** | **2-34** | 13 | 10 |
| E | G2024C | | | 7 | 0 | 26 | 11 | - | **1-40** | **25-34** | 10 | 10 |
| NS1 | A2434G | Ile5Val | - | 6 | 0 | 11 | 22 | - | **2-28** | **4-42** | 13 | 3 |
| NS1 | C2878A | Leu153Met | - | 9 | 0 | 16 | 33 | - | **24-35** | **17-44** | 10 | 17 |
| NS2A | G3629A | Arg51Lys | - | 11 | 0 | 26 | 33 | - | **1-48** | **1-48** | 18 | 14 |
| NS2A | C3875T | Ala133Val | - | 11 | 0 | 26 | 33 | - | **1-50** | **1-37** | 15 | 17 |
| NS2B | A4310G | Lys60Arg | - | 5 | 0 | 5 | 22 | - | 3 | **11-18** | 8 | 7 |
| NS3 | A6167G | Lys549Arg | - | 7 | 0 | 16 | 22 | - | **4-31** | **1-23** | 8 | 14 |
| NS3 | A6220G | Ile567Val | - | 9 | 0 | 16 | 33 | - | **4-35** | **7-45** | 13 | 14 |
| NS4A | A6491G | Lys39Arg | - | 10 | 0 | 11 | 44 | - | **10-38** | **3-42** | 15 | 14 |
| NS4A | T6496C | Tyr41His | Yes | 12 | 0 | 16 | 50 | - | **7-33** | **3-47** | 18 | 17 |
| NS4A | C6500A | Thr42Asn | - | 12 | 0 | 16 | 50 | - | **7-31** | **3-44** | 18 | 17 |
| NS4A | A6512G | Asn46Ser | - | 5 | 0 | 5 | 22 | - | 19 | **23-45** | 8 | 7 |
| NS4A | G6562A | Ala63Thr | Yes | 11 | 0 | 11 | 50 | - | **13-32** | **3-43** | 18 | 14 |
| NS4A | G6625A | Gly84Arg | Yes | 6 | 0 | 26 | 6 | - | 1 | 1 | 10 | 7 |
| NS4B | A6880G | Thr19Ala | Yes | 5 | 0 | 5 | 22 | - | 21 | 8-21 | 8 | 7 |
| NS5 | G8803A | Val412Ile | - | 10 | 0 | 16 | 39 | - | **1-31** | **25-48** | 18 | 10 |
| NS5 | G9501C | Gln644His | Yes | 9 | 0 | 21 | 28 | - | **3-47** | **2-38** | 13 | 14 |
| NS5 | A10075G | Ile836Val | - | 5 | 0 | 11 | 17 | - | 1-17 | **21-46** | 8 | 7 |
| NS5 | G10202A | Gly878Glu | Yes | 7 | 0 | 21 | 17 | - | 1-22 | **2-41** | 13 | 7 |
| NS5 | G10252A | Glu895Lys | Yes | 6 | 0 | 21 | 11 | - | **4-38** | **8-29** | 5 | 14 |
| 3UTR | 10389insAA | - | - | 9 | 0 | 11 | 39 | - | **2-29** | **3-30** | 15 | 10 |

| Subgroup 3 - WS+SD (5-11%, n=1-2 WS; 6-17%, n=1-3 SD) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | A187C | Lys31Gln | Yes | 3 | 0 | 11 | 6 | - | 3-4 | 1 | 8 | 0 |
| C | T235A | Phe47Ile | Yes | 2 | 0 | 5 | 6 | - | 2.3 | 1.4 | 5 | 0 |
| C | G406A | Val104Met | - | 2 | 0 | 5 | 6 | - | 13 | 22 | 3 | 3 |
| C | T431C | Val112Ala | - | 3 | 0 | 11 | 6 | - | **15-27** | **29** | 5 | 3 |
| prM/M | G481A | Gly15Ser | Yes | 3 | 0 | 5 | 11 | - | 5 | 3-11 | 3 | 7 |
| prM/M | A523G | Asn29Asp | Yes | 3 | 0 | 5 | 11 | - | **25** | **26-39** | 5 | 3 |
| prM/M | A555G | Ile39Met | - | 3 | 0 | 5 | 11 | - | 6 | 4-7 | 3 | 7 |
| prM/M | A731T | His98Leu | Yes | 2 | 0 | 5 | 6 | - | 1.5 | 1.1 | 3 | 3 |
| prM/M | G808A | Glu124Lys | Yes | 2 | 0 | 5 | 6 | - | 1 | 1 | 5 | 0 |
| E | G950C | Gly5Ala | - | 2 | 0 | 5 | 6 | - | 1.7 | 1 | 3 | 3 |
| E | G1039T | Ala35Ser | Yes | 2 | 0 | 5 | 6 | - | 1 | 6 | 5 | 0 |
| E | A1148C | Glu71Ala | - | 3 | 0 | 5 | 11 | - | 15 | 19-23 | 5 | 3 |
| E | A1207G | Ile91Val | - | 2 | 0 | 0 | 11 | - | - | **16-25** | 3 | 3 |
| E | G1543A | Asp203Asn | Yes | 4 | 0 | 5 | 17 | - | 5 | 4-16 | 5 | 7 |
| E | C1955T | Thr340Ile | Yes | 4 | 0 | 5 | 17 | - | 16 | 10-18 | 5 | 7 |
| E | T1972C | Tyr346His | Yes | 2 | 0 | 5 | 6 | - | 23 | **25** | 5 | 0 |
| E | T2065A | Tyr377Asn | Yes | 2 | 0 | 0 | 11 | - | - | 0.6-1 | 5 | 0 |
| E | A2105G | Asn390Ser | - | 2 | 0 | 5 | 6 | - | **38** | 19 | 3 | 3 |
| E | A2320G | Ile462Val | - | 4 | 0 | 5 | 17 | - | 14 | 7-10 | 5 | 7 |
| E | C2408T | Ala491Val | - | 2 | 0 | 5 | 6 | - | 6 | 10 | 0 | 7 |
| NS1 | A2989G | Asn190Asp | Yes | 3 | 0 | 5 | 11 | - | **41** | **21-34** | 5 | 3 |
| NS1 | G3057A | Met212Ile | - | 4 | 0 | 5 | 17 | - | 10 | 7-14 | 5 | 7 |
| NS1 | T3162A | Phe247Leu | Yes | 4 | 0 | 5 | 17 | - | 6 | **8-26** | 5 | 7 |
| NS1 | G3289A | Asp290Asn | Yes | 3 | 0 | 5 | 11 | - | 3 | 7-11 | 3 | 7 |
| NS2A | A3562G | Thr29Ala | Yes | 2 | 0 | 0 | 11 | - | - | 6-10 | 5 | 0 |
| NS2A | C3803T | Ala109Val | - | 2 | 0 | 5 | 6 | - | 3 | 9-15 | 3 | 3 |
| NS2B | G4456C | Val109Leu | - | 3 | 0 | 5 | 11 | - | 4 | 9-15 | 3 | 7 |

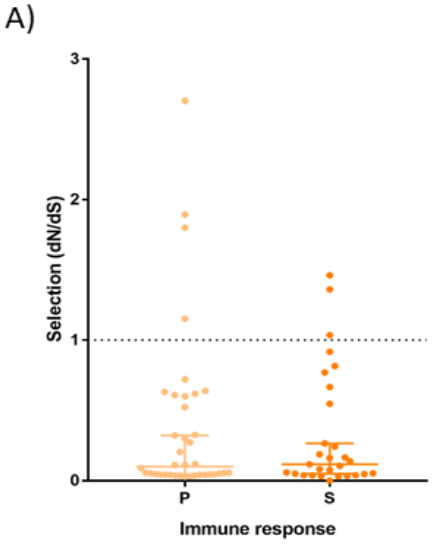
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NS2B | C4462G | Pro111Ala | Yes | 2 | 0 | 5 | 6 | - | 2 | 2 | 3 | 3 |
| NS2B | C4487T | Ala119Val | - | 2 | 0 | 5 | 6 | - | 13 | 11 | 0 | 7 |
| NS3 | G4829A | Gly103Glu | Yes | 3 | 0 | 11 | 6 | - | 1 | 0.8 | 8 | 0 |
| NS3 | A5078G | Lys186Arg | - | 3 | 0 | 0 | 17 | - | - | 6-12 | 5 | 3 |
| NS3 | A5092T | Met191Leu | - | 2 | 0 | 0 | 11 | - | - | 2-3.5 | 5 | 0 |
| NS3 | A5699T | Glu393Val | Yes | 2 | 0 | 5 | 6 | - | 1 | 2.5 | 5 | 0 |
| NS4B | C6884T | Thr20Ile | Yes | 2 | 0 | 0 | 11 | - | - | 16-20 | 3 | 3 |
| NS4B | G6889C | Glu22Gln | Yes | 4 | 0 | 5 | 17 | - | 13 | 6-15 | 5 | 7 |
| NS4B | T6892C | Ser23Pro | - | 4 | 0 | 5 | 17 | - | 14 | 7-15 | 5 | 7 |
| NS4B | G7186A | Val121Ile | - | 3 | 0 | 5 | 11 | - | 24 | **24-34** | 5 | 3 |
| NS5 | G7582A | Val5Ile | - | 3 | 0 | 5 | 11 | - | 23 | **7-41** | 5 | 3 |
| NS5 | A7777C | Met70Leu | - | 4 | 0 | 11 | 11 | - | **10-38** | **27-38** | 5 | 7 |
| NS5 | A8398T | Ile277Phe | Yes | 2 | 0 | 5 | 6 | - | 3.4 | 2.3 | 5 | 0 |
| NS5 | A8913T | Glu448Asp | - | 2 | 0 | 0 | 11 | - | - | 2 | 5 | 0 |
| NS5 | G9071A | Gly501Glu | Yes | 3 | 0 | 5 | 11 | - | 8 | 10-17 | 3 | 7 |
| NS5 | T9491C | Ile641Thr | Yes | 2 | 0 | 5 | 6 | - | 11 | 23 | 3 | 3 |
| NS5 | A9628G | Val687Ile | - | 3 | 0 | 11 | 6 | - | 1-14 | **46** | 5 | 3 |
| NS5 | C9857G | Thr763Ser | - | 2 | 0 | 0 | 11 | - | - | 5-7 | 3 | 3 |
| NS5 | A10205C | Tyr879Ser | Yes | 2 | 0 | 5 | 6 | - | 2 | 2 | 5 | 0 |
| 5UTR | A67T | - | - | 2 | 0 | 0 | 11 | - | - | 2-3 | 5 | 0 |
| 3UTR | A10369T | - | - | 2 | 0 | 0 | 11 | - | - | 4-14 | 3 | 3 |
| 3UTR | C10387T | - | - | 2 | 0 | 0 | 11 | - | - | 4-11 | 3 | 3 |
| 3UTR | 10387insAT | - | - | 2 | 0 | 0 | 11 | - | - | 6-17 | 3 | 3 |
| 3UTR | G10400A | - | - | 2 | 0 | 0 | 11 | - | - | 4-12 | 3 | 3 |
| 3UTR | C10411T | - | - | 2 | 0 | 0 | 11 | - | - | 4-16 | 3 | 3 |
| 3UTR | G10413A | - | - | 2 | 0 | 0 | 11 | - | - | 4-17 | 3 | 3 |
| 3UTR | C10450T | - | - | 3 | 0 | 0 | 17 | - | - | 1-12 | 5 | 3 |
| 3UTR | C10452T | - | - | 3 | 0 | 0 | 17 | - | - | 1-13 | 5 | 3 |
| 3UTR | 10611insA | - | - | 3 | 0 | 5 | 11 | - | **24** | 3-12.5 | 5 | 3 |

| Subgroup 4 - DF+WS+SD (Increasing/Decreasing inter-host frequency from DF to SD) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | G341T | Arg82Ile | Yes | 18 | 48 | 11 | 6 | 1-4 | 1-2 | 2 | 33 | 17 |
| E | T2210A | Leu425* | Yes | 16 | 32 | 26 | 6 | 1-2 | 1-4 | 1 | 28 | 17 |
| NS2A | G3928C | Ala151Pro | Yes | 19 | 52 | 11 | 6 | 1-2 | 2 | 2 | 36 | 17 |
| NS3 | G5026A | Glu169Lys | Yes | 7 | 16 | 5 | 6 | 1 | 1 | 7 | 15 | 3 |
| NS3 | T5304G | Cys261Trp | Yes | 11 | 26 | 11 | 6 | 1-2 | 1 | 1 | 13 | 21 |
| NS3 | G5531T | Arg337Ile | Yes | 21 | 45 | 21 | 17 | 1-4 | 1-5 | 1-5 | 44 | 14 |
| NS3 | C5717T | Ala399Val | - | 12 | 23 | 21 | 6 | 1-3 | 1-2 | 2 | 21 | 14 |
| NS4A | G6491A | Arg39Lys | - | 10 | 26 | 5 | 6 | 1-16 | 11 | **44** | 18 | 10 |
| NS4B | G6844A | Glu7Lys | Yes | 24 | 52 | 32 | 11 | 1-3 | 1-2 | 1 | 44 | 24 |
| NS4B | C6922T | Arg33Cys | Yes | 22 | 45 | 37 | 6 | 1-2 | 1-3 | 1 | 36 | 28 |
| NS4B | C6982T | Arg53* | Yes | 32 | 71 | 37 | 17 | 1-3 | 1-2 | 1-3 | 56 | 34 |
| NS4B | C7435G | Leu204Val | - | 8 | 16 | 11 | 6 | 0.5-1 | 1 | 1 | 15 | 7 |
| NS5 | G8074C | Glu169Gln | Yes | 20 | 55 | 11 | 6 | 1-3 | 1-2 | 1 | 36 | 21 |
| NS5 | A8932T | Met455Leu | - | 13 | 29 | 11 | 11 | 1-2 | 1-1.5 | 1 | 26 | 10 |
| NS5 | G9714C | Glu715Asp | - | 15 | 29 | 26 | 6 | 1-2 | 1 | 0.5 | 23 | 21 |
| NS5 | C9902G | Ala778Gly | - | 29 | 65 | 37 | 11 | 1-4 | 1-4 | 2-6 | 51 | 31 |
| NS5 | A10202G | Glu878Gly | Yes | 5 | 10 | 5 | 6 | **3-26** | 5 | **38** | 10 | 3 |
| 5UTR | G96A | - | - | 8 | 23 | 5 | 0 | 1-2 | 1 | 0 | 18 | 3 |
| 3UTR | 10389delA | - | - | 13 | 29 | 11 | 11 | **3-26** | 8-10 | **30-39** | 21 | 17 |
| 3UTR | C10408T | - | - | 10 | 23 | 5 | 11 | 1-18 | 6 | **15-32** | 18 | 10 |
| 3UTR | 10444insA | - | - | 6 | 13 | 5 | 6 | 2-4 | 6 | 6 | 10 | 7 |
| 3UTR | 10612insA | - | - | 5 | 13 | 5 | 0 | 1-7 | 4 | 0 | 8 | 7 |
| C | A127T | Thr11Ser | - | 6 | 6 | 5 | 17 | 5-9 | 7 | 14-17 | 5 | 14 |
| C | C431T | Ala112Val | - | 10 | 10 | 11 | 28 | 1-6 | 3-10 | **1-47** | 13 | 17 |
| prM/M | G523A | Asp29Asn | Yes | 8 | 6 | 11 | 22 | 1-5 | **9-35** | **1-36** | 10 | 14 |
| prM/M | C880T | His148Tyr | Yes | 6 | 3 | 11 | 17 | 1 | 2-14 | 8-24 | 5 | 14 |

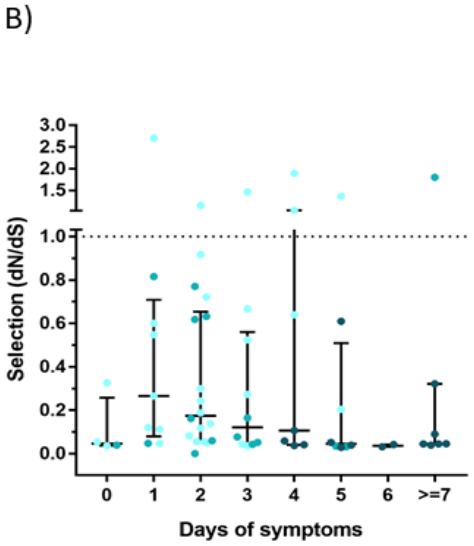
| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E | C1094T | Pro53Leu | Yes | 15 | 13 | 11 | 50 | 1-10 | 5-8 | **1-49** | 23 | 21 |
| E | A1214G | Lys93Arg | - | 9 | 6 | 11 | 28 | 6-9 | 4-21 | 11-20 | 13 | 14 |
| E | G1396A | Asp154Asn | Yes | 6 | 3 | 5 | 22 | 2 | **32** | 13-19 | 8 | 10 |
| E | A1858G | Ile308Val | - | 7 | 3 | 11 | 22 | 5 | 5-13 | **14-41** | 8 | 14 |
| E | C1972T | His346Tyr | Yes | 9 | 6 | 16 | 22 | 1-5 | **6-46** | **1-45** | 8 | 21 |
| E | C2276T | Ala447Val | - | 16 | 6 | 32 | 44 | 2-10 | **1-40** | **5-33** | 23 | 24 |
| NS1 | C2791T | Leu124Phe | Yes | 8 | 3 | 11 | 28 | 2 | 9-20 | 6-20 | 5 | 21 |
| NS1 | A2911T | Thr164Ser | - | 12 | 6 | 16 | 39 | 4-6 | **13-29** | **2-38** | 21 | 14 |
| NS1 | G2989A | Asp190Asn | Yes | 9 | 6 | 11 | 28 | 1-8 | 2-4 | **1-41** | 10 | 17 |
| NS2A | T3595C | Phe40Leu | Yes | 11 | 6 | 11 | 39 | 3-4 | 4-21 | **6-37** | 15 | 17 |
| NS2A | C3743T | Ala89Val | - | 5 | 3 | 5 | 17 | 4 | 12 | **2-27** | 10 | 3 |
| NS2A | G3874A | Val133Ile | - | 5 | 3 | 5 | 17 | 2 | 2 | 7-10 | 5 | 10 |
| NS2A | C3935T | Ser153Leu | Yes | 8 | 6 | 11 | 22 | 1-2 | 2-10 | 1-15 | 8 | 17 |
| NS2A | C3998T | Ala174Val | - | 8 | 6 | 5 | 28 | 1-2 | 12 | 1-18 | 10 | 14 |
| NS2A | T4009A | Ser178Thr | - | 6 | 3 | 5 | 22 | 2 | 11 | 8-19 | 8 | 10 |
| NS2A | A4034G | Gln186Arg | Yes | 16 | 6 | 26 | 50 | **8-25** | **1-25** | **1-46** | 28 | 17 |
| NS2A | A4069G | Ile198Val | - | 5 | 3 | 11 | 11 | 23 | 1-8 | 7-21 | 8 | 7 |
| NS2A | G4121A | Ser215Asn | - | 9 | 3 | 21 | 22 | 3 | 2-17 | 7-17 | 10 | 17 |
| NS2B | G4249A | Val40Ile | - | 13 | 6 | 26 | 33 | 2-3 | 8-14 | **6-26** | 23 | 14 |
| NS3 | G4604A | Arg28Lys | - | 9 | 3 | 11 | 33 | 4 | **6-38** | **1-25** | 15 | 10 |
| NS3 | A4739G | Lys73Arg | - | 5 | 3 | 11 | 11 | 2 | 6-24 | 17-24 | 5 | 10 |
| NS3 | A6203G | Lys588Arg | - | 5 | 3 | 11 | 11 | 2 | **3-28** | 10-12 | 3 | 14 |
| NS4A | G6512A | Ser46Asn | - | 8 | 6 | 11 | 22 | 1-12 | 7-14 | 2-15 | 8 | 17 |
| NS4A | A6652G | Ile93Val | - | 5 | 3 | 11 | 11 | 12 | **16-35** | 20-23 | 8 | 7 |
| NS4B | C6862T | Leu13Phe | Yes | 5 | 3 | 5 | 17 | 3 | 22 | 13-21 | 5 | 10 |
| NS4B | T6870G | Phe15Leu | Yes | 6 | 3 | 5 | 22 | 3 | **27** | 13-22 | 8 | 10 |
| NS4B | G6871A | Gly16Arg | Yes | 18 | 16 | 16 | 56 | 2-15 | 12-20 | **2-40** | 33 | 17 |
| NS4B | G6967A | Val48Ile | - | 7 | 3 | 11 | 22 | 2 | 2-10 | 1-16 | 8 | 14 |
| NS4B | A7186G | Ile121Val | - | 9 | 6 | 11 | 28 | 1-10 | 11-17 | **1-40** | 8 | 21 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NS5 | A8035G | Ile156Val | - | 5 | 3 | 5 | 17 | 1 | 10 | 4-13 | 5 | 10 |
| NS5 | G8204T | Arg212Leu | Yes | 7 | 3 | 21 | 11 | 2 | 1 | 1-1.5 | 13 | 7 |
| NS5 | A8242T | Thr225Ser | - | 6 | 3 | 5 | 22 | 3 | 5 | 1-23 | 8 | 10 |
| NS5 | A8306G | Lys246Arg | - | 8 | 3 | 11 | 28 | 6 | 5-15 | **1-25** | 10 | 14 |
| NS5 | G8572A | Val335Ile | - | 6 | 3 | 11 | 17 | 2 | **3-35** | 21-24 | 5 | 14 |
| NS5 | A9725T | Lys719Ile | Yes | 5 | 3 | 11 | 11 | 7 | 13-23 | 14-17 | 8 | 7 |
| NS5 | A10071T | Glu834Asp | - | 5 | 3 | 5 | 17 | 6 | 11 | **18-28** | 10 | 3 |
| NS5 | G10075A | Val836Ile | - | 8 | 6 | 5 | 28 | 1-9 | 19 | **2-31** | 10 | 14 |
| 3UTR | 10389insA | - | - | 18 | 16 | 21 | 50 | 1-21 | 1-20 | **1-36** | 26 | 28 |
| 3UTR | 10443insA | - | - | 11 | 3 | 21 | 33 | 0.6 | 2-4 | 2-3 | 18 | 14 |
| 3UTR | C10389T | - | - | 6 | 6 | 5 | 17 | 1-17 | 17 | 4-18 | 10 | 7 |

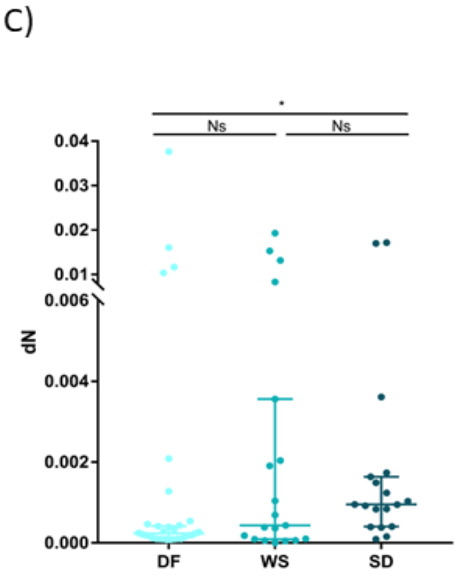
**Figure S6**— Natural selection (dN/dS) assessment for samples grouped by patient immune response (A) and patient days of symptoms (B). dN (C) and dS (D) analysed separetaly for samples grouped by clinical classification. Also, dN/dS ratio was computed for individual genes of the polyprotein within each sample and plotted for samples grouped by clinical classification, immune response or days of symptoms (E). Lines represent the median for each group, and error bars the Interquartile range. (*) p<0.01; (****) p<0.0001; Ns: not significant; DF: dengue fever; WS: dengue with warning signs; SD: severe dengue.



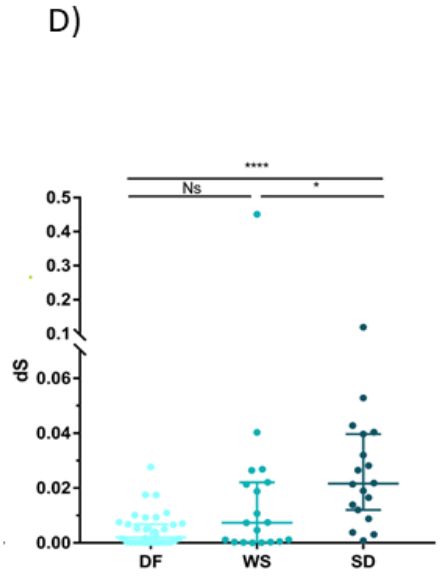

Mann-Whitney test for dN/dS vs immune response. P>0.05




Kruskal-Wallis test for dN/dS vs Days of symptoms. P>0.05

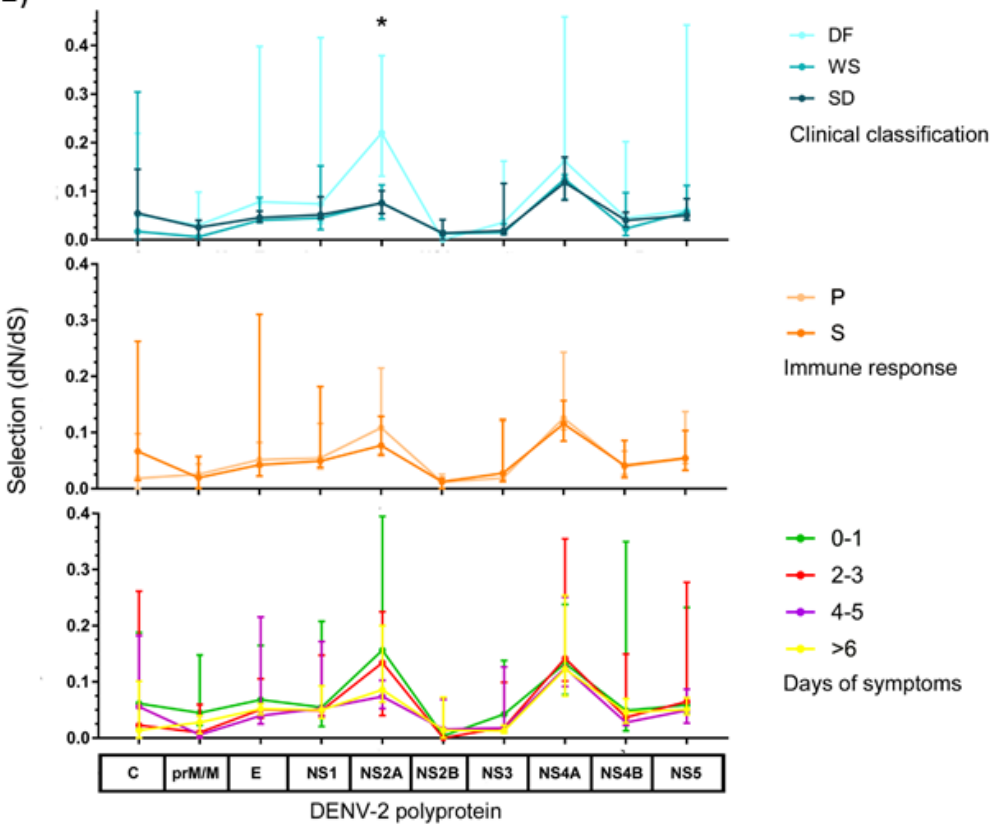



Kruskal-Wallis test for dN vs clinical classification; p=0.0478. Mann-Whitney test DFvsWS, p>0.05; DFvsSD, p=0.0048; WSvsSD, p>0.05




Kruskal-Wallis test for dS vs clinical classification; p=0.0001. Mann-Whitney test DFvsWS, p>0.05; DFvsSD, p<0.0001; WSvsSD, p=0.0172

E)




Kruskal-Wallis test for dN/dS medians comparison: DF clinical category, p=0.0005; WS clinical category, p=0.0051; SD clinical category; p=0.0001. (*) Mann-Whitney test, NS2A gene: DFvsWS p=0.0346; DFvsSD p=0.0134.

Kruskal-Wallis test for dN/dS medians comparison: P immune response category; p<0.0001; S immune response category; p=0.0043.

Kruskal-Wallis test for dN/dS medians comparison: 0-1 days of symptoms, p=0.0337; 2-3 days of symptoms, p<0.0001; 4-5 days of symptoms, p=0.0267; >6 days of symptoms; p=0.0331

**Table S7**—Immune response classification

| | Days from the onset of symptoms | | | |
|---|---|---|---|---|
| | ≤ 5 days | 6-9 days | ≥ 10 days | Immune response |
| IgG titer | < 160 | < 10240 | < 40960 | Primary |
| | ≥ 160 | ≥ 10240 | ≥ 40960 | Secondary |

| Sample | IgG titer[#] | DofS | Immune response |
|---|---|---|---|
| 137 | 2560 | 9 | Primary |
| 138 | < 40 | 2 | Primary |
| 139 | < 40 | 5 | Primary |
| 140 | 10240 | 10 | Primary |
| 141 | 160 | 5 | Secondary |
| 142 | < 40 | 5 | Primary |
| 143 | 40960 | ND | Secondary* |
| 144 | < 40 | ND | Primary* |
| 145 | 640 | 3 | Secondary |
| 146 | 160 | 2 | Secondary |
| 147 | 160 | 2 | Secondary |
| 148 | 640 | 1 | Secondary |
| 149 | 40 | 5 | Primary |
| 151 | 160 | 2 | Secondary |
| 153 | 2560 | 12 | Primary |
| 154 | 160 | 2 | Secondary |
| 155 | 640 | 2 | Secondary |
| 156 | < 40 | 3 | Primary |
| 157 | 2560 | 3 | Secondary |
| 158 | 160 | 1 | Secondary |
| 159 | 160 | 3 | Secondary |
| 160 | 160 | 0 | Secondary |
| 161 | 40 | 3 | Primary |
| 162 | 40 | 2 | Primary |
| 163 | 640 | 2 | Secondary |
| 166 | <40 | 1 | Primary |
| 167 | <40 | 3 | Primary |
| 168 | 160 | 2 | Secondary |
| 169 | <40 | 2 | Primary |
| 170 | 40 | 1 | Primary |
| 171 | 40 | 1 | Primary |
| 172 | 40 | 2 | Primary |
| 173 | 640 | 4 | Secondary |
| 174 | 40 | 3 | Primary |
| 175 | 160 | 1 | Secondary |
| 177 | <40 | 4 | Primary |
| 178 | <40 | 4 | Primary |
| 179 | <40 | 0 | Primary |

| | | | |
|---|---|---|---|
| 180 | <40 | 2 | Primary |
| 181 | 640 | 2 | Secondary |
| 182 | <40 | 2 | Primary |
| 183 | 40 | 1 | Primary |
| 184 | <40 | 0 | Primary |
| 185 | 640 | 2 | Secondary |
| 186 | 40 | 3 | Primary |
| 187 | 160 | 3 | Secondary |
| 188 | <40 | 2 | Primary |
| 189 | <40 | 1 | Primary |
| 190 | 640 | 2 | Secondary |
| 191 | 640 | 0 | Secondary |
| 192 | 640 | 2 | Secondary |
| 193 | 40 | 3 | Primary |
| 194 | <40 | 4 | Primary |
| 195 | 40 | 4 | Primary |
| 196 | 40 | 5 | Primary |
| 197 | 2560 | 7 | Primary |
| 198 | <40 | 6 | Primary |
| 199 | 40960 | 7 | Secondary |
| 200 | 40960 | 4 | Secondary |
| 201 | 2560 | 4 | Secondary |
| 202 | 40 | ND | Primary* |
| 203 | <40 | 5 | Primary |
| 204 | 640 | 8 | Primary |
| 205 | 160 | 1 | Secondary |
| 206 | 2560 | 5 | Secondary |
| 207 | 640 | 5 | Secondary |
| 208 | 10240 | 24 | Primary |
| 209 | 2560 | 6 | Primary |

DofS: days since the onset of symptoms; ND: not determined. #Determined by an in-house ELISA assay. *Even though cases 143, 144 and 202 lack the information of patient days of symptoms, immune response could be anyway determined because IgG titer resulted as low or high as needed, respectively, to classify the immune response independently to the days of symptoms. This peculiarity allowed theses cases to be included in the study.

**Table S8**—GenBank accession numbers of the DENV-2 sequences employed in the phylogenetic analysis

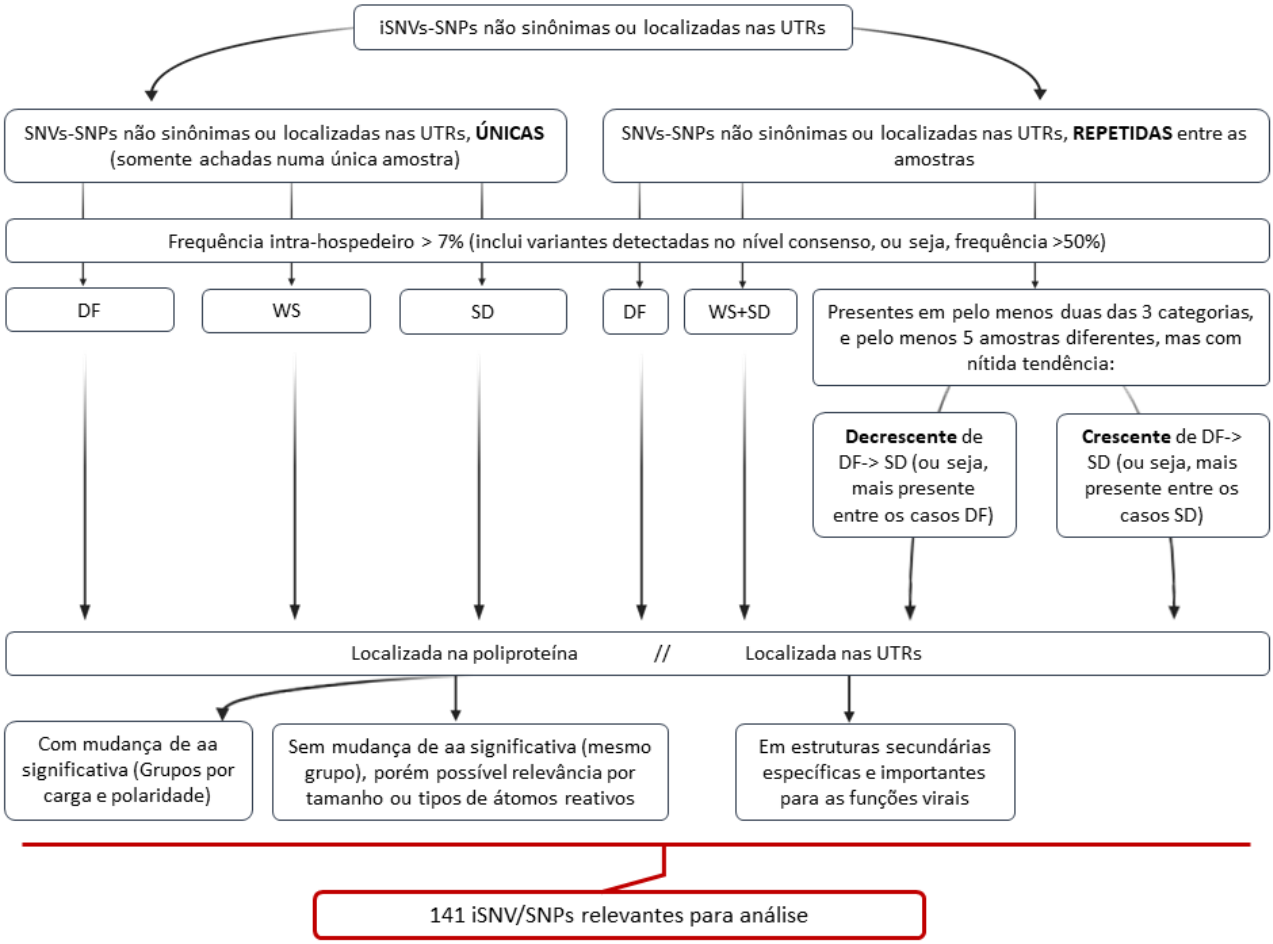
| | | | | | | |
|---|---|---|---|---|---|---|
| AF208496 | EU920828 | FJ850107 | GU131902 | JN819424 | KC294221 | KY474328 |
| AB122022 | EU920829 | FJ850108 | GU131947 | JX286516 | KC294222 | KY474329 |
| AY702035 | EU920830 | FJ850112 | GU131955 | JX286518 | KC294223 | KY474330 |
| AY702036 | EU920831 | FJ850119 | GU131959 | JX286519 | KF955360 | KY474331 |
| AY702037 | EU920832 | FJ850120 | GU131974 | JX286520 | KF955385 | KY474332 |
| AY702038 | EU920833 | FJ873808 | GU131975 | JX286521 | KF955395 | KY474333 |
| AY702039 | EU920834 | FJ898438 | GU369819 | JX286522 | KJ189305 | KY474334 |
| DQ181804 | EU920835 | FJ898439 | HM181971 | JX286523 | KJ189308 | KY627762 |
| DQ181805 | EU920836 | FJ898450 | HM582117 | JX286524 | KJ189309 | KY921904 |
| EU056811 | EU920837 | FJ898451 | HM631865 | JX286525 | KJ189310 | KY921905 |
| EU482444 | EU920838 | FJ898452 | HQ012533 | JX286526 | KJ189311 | LC436674 |
| EU482544 | EU920840 | FJ898453 | HQ012534 | JX567950 | KJ189370 | MF459663 |
| EU482545 | EU920841 | FJ898460 | HQ012535 | JX567951 | KP188549 | MH069495 |
| EU482548 | EU920842 | FJ898461 | HQ012536 | JX669476 | KP188550 | MH069496 |
| EU482550 | EU920843 | FJ898465 | HQ012537 | JX669477 | KP188551 | MH069497 |
| EU482556 | EU920844 | FJ898466 | HQ012538 | JX669478 | KP188552 | MH069498 |
| EU482560 | EU920845 | FJ898467 | HQ026763 | JX669479 | KP188553 | MH069499 |
| EU482601 | EU920846 | FJ898478 | HQ332184 | JX669480 | KP188554 | MH215277 |
| EU482603 | EU920847 | FJ906959 | HQ332185 | JX669481 | KP188555 | MH613984 |
| EU482604 | EU920848 | GQ199890 | HQ332186 | JX669482 | KP188556 | MH613985 |
| EU482605 | EU920849 | GQ199892 | HQ332187 | JX669483 | KP188569 | MH613986 |
| EU482606 | FJ024473 | GQ199893 | HQ332188 | JX669484 | KU509267 | MH781013 |
| EU482607 | FJ024474 | GQ199894 | HQ332189 | JX669485 | KU509273 | MH781014 |
| EU482608 | FJ024475 | GQ199895 | HQ332190 | JX669486 | KU509274 | MH781015 |
| EU482723 | FJ024477 | GQ199898 | HQ541786 | JX669487 | KX452046 | MH822951 |
| EU482725 | FJ182012 | GQ868540 | HQ541787 | JX669488 | KX702403 | MH823208 |
| EU482726 | FJ639705 | GQ868549 | HQ541788 | KC294200 | KX702404 | MH888331 |
| EU482731 | FJ639732 | GQ868551 | HQ541792 | KC294201 | KY415992 | MK268692 |
| EU482734 | FJ639733 | GQ868552 | HQ541793 | KC294202 | KY474308 | MK411559 |
| EU482735 | FJ639734 | GQ868553 | HQ541794 | KC294203 | KY474309 | MK517773 |
| EU482755 | FJ639783 | GQ868554 | HQ541798 | KC294204 | KY474312 | MN239505 |
| EU569708 | FJ639788 | GQ868555 | HQ705624 | KC294205 | KY474313 | MN272404 |
| EU596488 | FJ639809 | GQ868556 | HQ733861 | KC294206 | KY474314 | MN272405 |
| EU596490 | FJ639822 | GQ868557 | HQ999999 | KC294207 | KY474315 | MN959474 |
| EU596491 | FJ850050 | GQ868558 | JF327392 | KC294208 | KY474316 | MN959475 |
| EU677142 | FJ850060 | GQ868596 | JF357906 | KC294209 | KY474317 | MN959476 |
| EU677145 | FJ850072 | GQ868603 | JF730051 | KC294210 | KY474318 | MN959477 |
| EU687212 | FJ850074 | GQ868604 | JF730053 | KC294211 | KY474319 | MN959478 |
| EU687216 | FJ850076 | GQ868640 | JF730054 | KC294212 | KY474320 | MN959479 |
| EU687217 | FJ850078 | GQ868641 | JN819404 | KC294213 | KY474321 | MN959480 |
| EU687220 | FJ850082 | GU131864 | JN819407 | KC294214 | KY474322 | MN959481 |
| EU687230 | FJ850085 | GU131881 | JN819408 | KC294216 | KY474323 | MT076937 |
| EU687241 | FJ850088 | GU131882 | JN819416 | KC294217 | KY474324 | MT897896 |
| EU687245 | FJ850091 | GU131883 | JN819419 | KC294218 | KY474325 | MT899084 |
| EU726775 | FJ850105 | GU131884 | JN819421 | KC294219 | KY474326 | MT899085 |
| EU854294 | FJ850106 | GU131885 | JN819422 | KC294220 | KY474327 | |

## 4.3 Análises das mutações consideradas relevantes

Conforme os resultados obtidos anteriormente, este último eixo do projeto resulta da busca de pontos específicos do genoma viral submetidos a maior mutagênese e possam de alguma forma estar relacionados à clínica desenvolvida pelos pacientes, assim como também da análise pontual de mutações virais consideradas de relevância.

Dentre as 1123 diferentes variantes de caráter não sinônimo ou localizadas nas regiões não codificantes do genoma viral, apenas 141 foram selecionadas para análises subsequentes. Os critérios tidos em conta incluíram: i) si se tratava de variantes únicas ou achadas repetitivamente entre as amostras; ii) categoria clínica na qual a variante foi achada; iii) frequência da variante maior ou igual a 7%, incluindo-se aqui os polimorfismos detectados no nível consenso (frequência alélica > 50%); iv) localização em genes codificantes ou em estruturas secundárias conhecidas nas UTRs do RNA; v) mudança de aminoácido envolvida (Figura 4.2).

**Figura 4.2** Fluxograma para seleção de variantes de relevância




Notas: Aa: amino acido; DF/DWS/SD: categorias da classificação clínica (WHO, 2009) que incluem os casos clássicos de febre da dengue, os casos de dengue com sinais de alarme, e os casos de dengue grave, do inglês “dengue fever/dengue with warning signs/severe dengue”; iSNV: variantes de nucleotídeo único intra-hospedeiro, do inglês “ intrahost single nucleotide variant”; SNP: polimorfismos de nucleotídeo único, referindo-se às variantes detectadas no nível consenso, do inglês “single nucleotide polymorphism”; UTRs: regiões não codificantes do genoma viral, do inglês “untranslated regions” (se mantêm a mesma nomenclatura dos artigos para maior claridade).

Desta forma, no seguinte artigo se avaliou a presença de pontos quentes mutacionais (“hotspots”) no genoma viral e o possível efeito das variantes selecionadas sobre a estrutura e função, tanto das proteínas viras quanto das estruturas secundárias nas UTRs.

**4.3 Artigo 3** - In-silico analysis of Dengue virus serotype 2 mutations detected at intrahost level in patients with different clinical outcomes

**Relação do manuscrito com os objetivos:** Os resultados apresentados neste manuscrito são referentes aos seguintes objetivos específicos:

4) Determinar se existem pontos quentes mutacionais (do inglês “hotspots”) em alguma região do genoma viral em particular de acordo com a classificação do quadro clínico;

5) Construir modelos em três dimensões das diferentes proteínas virais, e determinar qual o impacto das substituições de nucleotídeo único achadas consistentemente nos espectros de mutantes das diferentes amostras sobre a estrutura das mesmas, inferindo possíveis mudanças na estrutura e função das mesmas;

6) Determinar qual o impacto das substituições de nucleotídeo único achadas sobre as estruturas secundárias de RNA existentes nas regiões não codificantes do genoma viral, inferindo possíveis impactos estruturais e no *fitness* viral.

**Situação do manuscrito:** Artigo aceito na revista *Microbiology Spectrum*.

**Fator de Impacto da Revista**: 6,62.

**In-silico analysis of Dengue virus serotype 2 mutations detected at the intrahost level in patients with different clinical outcomes**

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## Abstract

Intrahost genetic diversity is thought to facilitate arbovirus adaptation to changing environments and hosts, and it may also be linked to viral pathogenesis. Intending to shed light on the viral determinants for severe dengue pathogenesis, we previously analyzed the DENV-2 intrahost genetic diversity in 68 patients clinically classified as dengue fever (n=31), dengue with warning signs (n=19), and severe dengue (n=18), performing viral whole-genome deep sequencing from clinical samples with an amplicon-free approach. From it, we identified a set of 141 relevant mutations distributed throughout the entire viral genome that deserved further attention. Therefore, we employed molecular modelling to recreate three-dimensional models of the viral proteins and secondary RNA structures to map the mutations and assess their potential effect. Results showed that, in general lines, disruptive variants were primarily identified among dengue fever cases. In contrast, potential immune-escape ones were mainly associated with warning signs and severe cases, in line with the latter's longer intrahost evolution times. Furthermore, several mutations were located on protein-surface regions, with no associated function. They could represent sites of further

investigation, as the interaction of viral and host proteins is critical for both host immunomodulation and virus hijacking of the cellular machinery. The present analysis provides new information about the implications of the intrahost genetic diversity of DENV-2, contributing to the knowledge about the viral factors possibly involved in its pathogenesis within the human host. Upon strengthening our results with functional studies, many of these variants could be considered in the design of therapeutic or prophylactic compounds and the improvement of diagnostic assays.

**Importance**

Previous evidence showed intrahost genetic diversity in arboviruses may be linked to viral pathogenesis and that one or a few amino acid replacements within a single protein are enough to modify a biological feature of an RNA virus. To assess dengue virus 2 determinants potentially involved in pathogenesis, we previously analyzed its intrahost genetic diversity in patients with different clinical outcomes and identified a set of 141 mutations that deserved further study. Thus, through a molecular modeling approach, we showed that disruptive variants were primarily identified among cases with mild dengue fever, while potential immune-escape variants were mainly associated with cases of greater severity. We believe that some of the variants pointed out in this study resulted attractive enough to be potentially considered in future intelligent designs of therapeutic or prophylactic compounds or the improvement of diagnostic tools. The present analysis provides new information about DENV-2 viral factors possibly involved in its pathogenesis within the human host.

**Introduction**

Dengue virus (DENV), a member of the genus *Flavivirus* and family *Flaviviridae,* is a significant emerging arthropod-borne pathogen infecting millions worldwide. DENV infection can range from asymptomatic infection to a debilitating and potentially life-threatening acute disease in human hosts (1).

DENVs are positive-sense, single-stranded-RNA viruses (2). Their genome's size is approximately 10.7 kb and contains a region coding for a single polyprotein flanked by a short 5' untranslated region (UTR) and a longer 3' UTR highly structured and carrying elements essential to the virus replication (2). The polyprotein is post-

translationally cleaved into three structural proteins: Capsid (C), pre-Membrane/Membrane (prM/M), and Envelope (E); and seven non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (2). Overall, the structural proteins mediate virus attachment (E), entry (E and prM/M), assembly (C), and secretion (prM and E). In contrast, the NS proteins carry mainly enzymatic activities (NS3, NS2B3, and NS5) or essential roles in the replication complex assembly, plus host immune-response modulation properties (NS1, NS2A, NS2B3, NS4A, NS4B) (2). The NS3 protein has different domains associated with distinct functions: helicase, RNA 5' triphosphatase, and nucleoside triphosphatase within its C-terminal domain, and serine protease within its N-terminal domain (3), activity for which association with NS2B is essential (NS2B3, cofactor–protease complex) (4). NS5 possesses methyltransferase and guanylyltransferase activities within its N-terminal domain while encoding for the replicative RNA-dependent RNA polymerase (RdRp) within its C-terminal domain (5). The latter is a low-fidelity enzyme and is thus prone to introducing genetic variability into the viral population during each RNA replication cycle. Consequently, new viral variants are continuously generated within a single host, shaping what has been defined as 'intrahost diversity' (6).

However, viral proteins were demonstrated to be, in fact, multi-functional, also playing different roles in short-circuiting functional pathways of the host cell (7), which is not surprising considering the extremely small viral proteomes. This multi-functionality has been attributed to the viral proteins' structural uniqueness, which often contains functional intrinsically disordered regions (8-9).

Intrahost genetic diversity is thought to be advantageous for RNA viruses by facilitating their adaptation to changing environments and hosts, and influencing their pathogenicity (10-13). Previous studies have shown that just one or a few amino acid replacements within a single protein are enough to modify a particular biological feature of an RNA virus (14, 15). Besides, several in vitro mutagenesis analyses performed on DENV proteins have proven how particular point-mutations modify their activity' efficacy, ultimately impacting viral replication, immune system regulation, and viral fitness (16-18). Considering all the above-mentioned, and to better understand the association of viral features with severe dengue pathogenesis, we have previously explored DENV-2 intrahost genetic diversity in 68 Brazilian patients with different

clinical outcomes with an amplicon-free deep-sequencing experimental approach. On these patients' mutant swarms, we looked for any mutational pattern that could be correlated with the disease's clinical outcome and detected a set of 141 intrahost single-nucleotide variants (NS-iSNV) and single nucleotide polymorphisms (SNP; variants detected at consensus level, i.e., allele frequency higher than 50%) located along the viral genome, that were consistently identified among the samples and were worthy for in-deep analysis (19). Therefore, to determine whether there is a potential structural or functional significance for these minor variants, we sought to assess their effect on viral proteins or RNA secondary structure employing molecular modelling techniques. Besides, under the assumption that recurrently mutated genomic regions, commonly known as hotspots, might be presumably functional and could help us understand evolutionary mechanisms that might impact virulence (20), we studied the presence of potential hotspots throughout the DENV-2 genome considering the entire mutational dataset determined in our previous work, i.e., 10180 insertions/deletions, synonymous and nonsynonymous substitutions (19).

## Methodology

### Ethical statement

This study was approved by the Oswaldo Cruz Institute Ethical Committee in Research (CAAE 90249219.6.1001.5248 number 2.998.362). All methods were performed in accordance with the World Medical Association Declaration of Helsinki.

### Study samples

Sixty-eight serum samples of DENV-2 confirmed cases from the Brazilian states of Rio de Janeiro, São Paulo, and Minas Gerais, collected between 2007 and 2019, were included in our study. The cases' clinical classification was performed according to the 2009 World Health Organization guidelines (1), and samples were processed, deep-sequenced and analyzed as previously described (19). All NGS can be accessed from the NCBI BioProject PRJNA541495.

### Variants’ dataset

A dataset of 141 iSNVs (detected at intrahost level) and SNPs (detected at consensus level), was defined for analysis (Supplementary Table S1). Criteria for variants' inclusion in the dataset involved: i) exclusively found among cases with dengue fever (DF) or cases with warning signs (WS) + severe cases (SD); ii) detected in the three clinical categories at highly interhost frequency (n=5-19), but with a marked tendency of increasing/decreasing interhost frequency from DF to WS+SD cases; iii) non-conservative amino acid substitution or localized in the UTRs; iv) intrahost frequency ≥ 7%.

Protein structure prediction

The structural impact of the selected NS-iSNV and SNPs was assessed by constructing three-dimensional (3D) models using different methodologies. Firstly, a multiple sequence alignment with the translated consensus sequences was performed using ClustalW (https://embnet.vital-it.ch/software/ClustalW.html) and Cd-hit (https://cd-hit.org/) servers to identify and eliminate redundancy. One representative sequence of each cluster with 100% identity was selected (Supplementary Table S2). Next, local sequence alignments were performed to define templates for the modelling process. The template structures were selected by Advanced Search using the Protein Data Bank (PDB) (http://www.pdb.org). The PDB search score, R-value, resolution, identity and organism were employed as the selection criteria. Ligands in the active sites were also taken into consideration when relevant.

a. Comparative Modelling

Homologous structures were found for C, prM, E, NS1, NS3 and NS5 proteins, making it possible to implement a comparative modelling strategy (Supplementary Table S3). Protein models were constructed using the MODELLER software v9.25 (21). The previously defined template and target sequences were used as the software's input information (scripts for model generation available at https://github.com/mpds/denv-scripts). Five different models were built for each sequence, and selection of the best model for each target was made according to DOPE score (Discrete optimized protein energy score, obtained from MODELLER's model assessment) and visual inspection performed on PyMol v2.4.1 (https://pymol.org/2/).

b. Template-free modelling

The alignment analyses performed with Blastp returned no PDB matches against the representative sequences of NS2A, NS2B, NS4A and NS4B proteins suitable for comparative modelling uses. Thus, different strategies were implemented to predict the functional structure of these targets: (i) threading, by I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) (22), (ii) secondary structures prediction using Psipred (23), (iii) transmembrane protein topology prediction by Mem-sat (24), and de novo prediction, using DMPfold (http://bioinf.cs.ucl.ac.uk/index.php?id=780) (25). Results were inspected using the PyMol program and compared to theoretical models proposed for NS2A (26), NS2B (27), NS4A (28) and NS4B (29), respectively.

Model quality assessment

The model's stereochemical properties were estimated using PROCHECK (30), ERRAT (31) and VERIFY 3D (32), through SAVES 6.0 server (https://saves.mbi.ucla.edu/). PROCHECK results were mainly considered for models' quality assessment, leaving the ERRAT and VERIFY 3D results as secondary evaluation criteria. The Ramachandran plots, which provide an insight into the possible combinations of torsion angles of amino acids residues, were also inspected and validated against the respective template's crystallographic information when available. The template-free models were evaluated by the expected parameters of suitable protein structures.

iSNV/SNP effect assessment

PROVEAN server's tool (http://provean.jcvi.org/index.php) (33) was employed as an auxiliary tool to predict the effect of the selected iSNV/SNPs on the structure and function of the viral proteins. It uses alignment scores to estimate the impact of sequence variation on the biological function of proteins. Furthermore, PyMol v2.4.1 was employed for visual inspection and an in-depth analysis of the mutations' potential effect, also checking possible secondary structural changes. All models were compared to their experimentally resolved templates (when available) to ultimately gain knowledge on how the variants under investigation can influence the structure, dynamics and consequently function of the studied proteins.

Molecular docking

Potential interference caused by variants located within active sites ou ligand-binding sites were determined with molecular docking experiments using Glide software (Schrödinger Release 2021-1: Glide, Schrödinger, LLC, New York, NY, 2021) (34). Before grid generation and docking, the protonation states and protein' H-bonds networks were optimised with the Protein Preparation Wizard tool (35), of the Maestro software suite v12.4 (Schrödinger Release 2021-1: Maestro, Schrödinger, LLC, New York, NY, 2021), considering the pH information at the template-crystallization procedure (35). When possible, small molecules (obtained from templates, by comparative modelling) were preserved at their respective binding sites.

Two docking experiments were carried out within this study. The docking parameters for the C protein analysis were defined by the redocking studies with crystal 6VG5 structure. Initially, all structures here modelled were aligned to 6VG5 and parameters obtained were extrapolated to all structures. The employed outer box was 39Â in the three dimensions, centered in 18.05x, 6.28y, 29.68z. The inner box was defined in 10XYZ. Also, the extra precision method and OPLS_2005 force field were used. For NS3 protein, the same approach was used to define the grid box, however, with unsuccessful results. Thus, the 261 and 263 residues were defined as the center of the cavity under study (see below) in the HDOCK server (http://hdock.phys.hust.edu.cn/) (36), to apply then the template-based methodology.

RNA secondary structure assessment

Secondary RNA structures on both 5' and 3' UTR, plus alterations caused by variants here analyzed, were inferred with RNAfold server (http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi), which predicts minimum free energy structures and base pair probabilities from single-stranded RNA.

Since these two highly structured regions are already well-described for DENV (37-39), each secondary structure's coordinates' information within these regions was obtained from the literature, and they were separately analyzed. Samples 145 and 137 were considered baselines for 5' and 3' UTR modelling, respectively, since they represent most of the samples' sequences.

The optimal secondary structure with a minimum free energy (MFE) and thermodynamic ensemble characteristics (the diversity and the free energy of the thermodynamic ensemble, the frequency of the MFE structure in the ensemble, and

the centroid secondary structure with MFE, representing the structure with the minimum total base pair distance to all structures in the ensemble) were predicted for each modelled structure (40), either the baselines or the variants' carriers. iSNVs were considered in their respective consensus-sequence environment.

Mutational burden analysis

Recurrently mutated genomic regions, also known as hotspots, are relevant because they are presumably functional and could help us understand evolutionary mechanisms that might impact virulence. In this work, we employed a mutation burden analysis by comparing the mutational density of different fixed-length regions, throughout the virus' entire genome, between data with different disease clinical classifications. Additionally, a binomial test of significance was applied to mutation data within each class, in order to identify those regions with a number of mutated positions above the background expectations, i.e., possible hotspot candidates (20).

Genome variant data from all the 68 samples' intrahost populations, including indels, synonymous and nonsynonymous substitutions, were considered according to Torres et al, 2021 (19). The data were grouped into three classes following clinical classifications (DF, WS, and SD), and the unique variants found were filtered for each category. Mutational density was calculated as $x_i / n$, where $x_i$ is the total number of unique mutations found in the $i$-th region, with a fixed length equal to $n$ bases, for $i = 1, ..., k$ for all $k$ different regions considered. Regions were defined by sliding a fixed-length, non-overlapping window over the genome positions. In our tests, we used different window sizes of 9, 12, 15, and 18 (multiples of three, a codon's size).

For each genomic region, defined as above, a binomial test was used to identify hotspot candidates. This approach assumes that the mutation rate within the given region is constant and mutations occur independently. A $p$-value was then calculated for each region, taking into account its length, mutation count, and a background mutation rate. The background mutation rate $p$ was considered separately for each gene (and 3' UTR and 5' UTR regions), calculated as the total number of mutations in the gene divided by its size. Regions falling inside a particular gene used the respectively calculated $p$. If the region happened to span over two different genes, a global background rate was considered. All $p$-values were adjusted for multiple testing using the Benjamini-Hochberg method. Analyses were performed using Python

version 3.8.5, and the respective scripts are available at https://github.com/mpds/denv-scripts.

## Results and discussion

Intrahost genetic diversity has been demonstrated for RNA viruses to be advantageous, facilitating their adaptation to different environments and hosts (10-13). Also, it can significantly contribute to viral pathogenesis, allowing the modulation of the expression of distinct phenotypic characteristics (41, 42), the escape to immune pressures, and the development of rapid resistance to vaccines and antiviral drugs (6). Considering that one or a few amino acid replacements within a single protein are enough to modify a biological feature of a virus (14, 15), the intrahost diversity takes a place of high relevance on the study of DENV evolution during human infection and its relation with disease severity. Therefore, to better understand the association of viral features with severe dengue pathogenesis, we have previously explored DENV-2 intrahost genetic diversity in 68 Brazilian patients with different clinical outcomes with an amplicon-free deep-sequencing experimental approach. We looked for any mutational pattern that could be correlated with the disease's clinical outcome, and we detected a set of 141 iSNVs + SNPs located along the viral genome that were consistently identified among the samples and were worthy for in-deep analysis (19). Thus, to determine whether there is a potential structural or functional significance for these minor variants, we assessed their effect on viral proteins employing molecular modeling techniques. For structural (C, prM and E) and three non-structural proteins (NS1, NS3 and NS5), a comparative-modelling strategy was implemented since templates for these targets were already available in open databases. Characteristics of each model can be found in Supplementary Table S4. Finally, DENV-2 variants previously detected within the 68 patients with different clinical outcomes (Supplementary Table S1) (19) were mapped on their respective models, and their effects were assessed by visual inspection in PyMol v9.25 (https://pymol.org/2/) and molecular docking techniques. On the other hand, for viral proteins NS2A, NS2B, NS4A and NS4B, a fold recognition and a de novo approach were employed instead, which will be addressed below.

Structural proteins

*Capsid*

Based on previous studies, C model was constructed as a monomer subunit of the functional homodimer, as displayed in blue in Fig 1 (43, 44). Even though C is the least genetically conserved among Flavivirus proteins, its structure and charge distribution do, with alpha-helices a2 and a3 conforming a hydrophobic region involved in membrane interaction and the highly basic a4 in RNA interaction (43). This asymmetric charge distribution was conserved also in crystal structure 6VG5, employed as a template for model construction, and thus in our C model. However, since it only covered residues 21 to 100, three variants mapping into residues 10 and 104 could not be analyzed by this approach. Substitutions on residue 10 detected at consensus level on a WS case (S10I) and as minor variant at intrahost level on 3 DF cases (S10N) were located in the N-terminal region of C, a 20-residue tail conformationally labile but highly basic (43). Thus, considering Serine's substitutions for Isoleucine and Asparagine, respectively, they would not be expected to cause any severe alteration to C properties and function, as predicted by PROVEAN as well. Also, substitution V104M, although detected at intrahost level in one WS and one SD case, would not cause any disruption on the mature C protein because the C-terminal

hydrophobic tail is removed after NS2B-NS3 protease cleavage (43). Furthermore, the substitution of Valine for Methionine would not significantly alter its hydrophobicity.

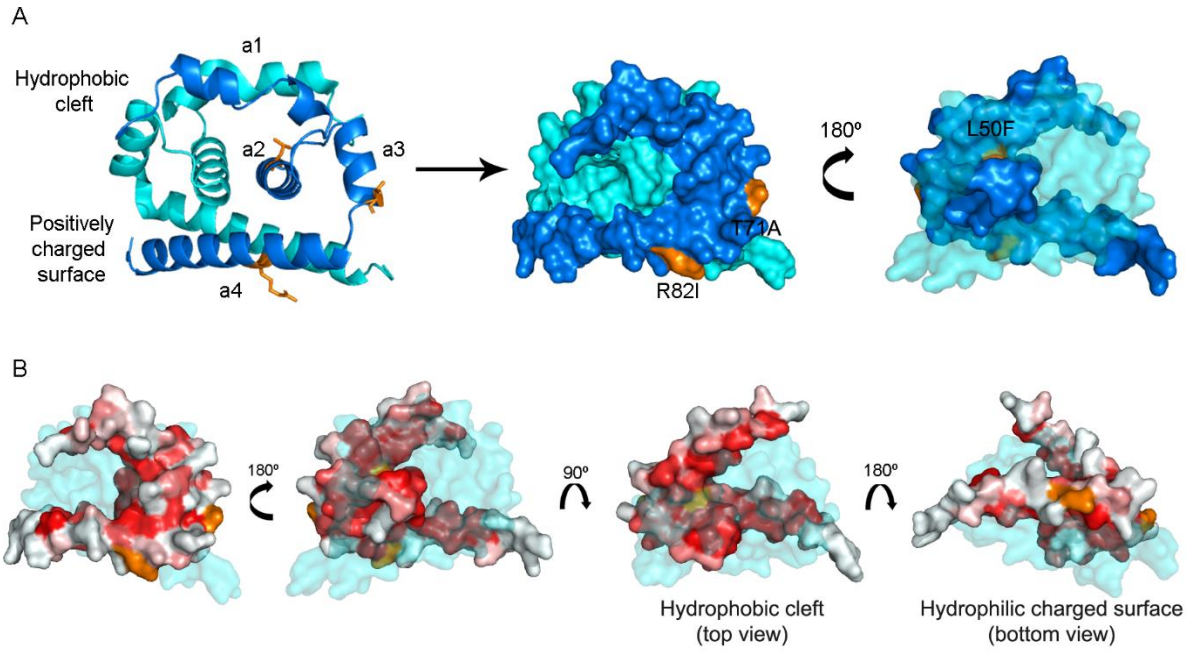



**FIG 1** Structure of DENV-2 C, residues 21-100. A) The cartoon diagram shows the homodimer with the determined secondary alpha-helices in the left panel, with the blue chain obtained by modelling and the light blue belonging to template 6VG5. In the right panel, the same homodimer's surface diagram. Mutations' locations and residues are denoted in orange. B) Hydrophobicity pattern over the monomer surface, in a colored-scale varying from white (highly hydrophilic, score: -2.53) to red (highly hydrophobic, score:1.38) based on Eisenberg's hydrophobicity scale (43).

On the contrary, residues 50, 71 and 82, denoted in orange in Fig 1, are located within alpha-helices 2, 3 and 4, respectively. Substitution L50F was detected at consensus level in 2 DF cases. Even though it is not a residue involved in the dimer interface because of its side chain's orientation (43), the substitution of Leucine for Phenylalanine at position 50 might introduce a side-chain residue with a higher volume. Still a hydrophobic residue, it would not be expected to impair membrane interaction. Mutation L50S has demonstrated to alter C accumulation significantly on lipid droplets, resulting in the attenuation of viral particles production (46). It is unclear whether a similar situation could be involved in the mild disease clinical outcome of these two cases, but it could be a suitable explanation. The possible interferences of the L50

substitutions were also evaluated through docking studies, since template 6VG5 used for modelling was co-crystallized in complex with a protein inhibitor, and residue L50 was located within the interacting region. This inhibiting compound is incorporated into virions by inducing capsid tetramerization, leading to virions that are defective in nucleocapsid uncoating when infecting new cells (44). Firstly, redocking experiments were performed with the 6VG5 complex to validate the experiments and the preparation step. The experimental binding of the ST148 inhibitor observed in the 6VG5 complex, occurred in a cavity defined by residues T30, F33, L35, M37, L38, L50, and F53, at the interface between the two capsid monomers. Two H bonds linked the amine group of the ST148 with F33 main-chain, while hydrophobic interactions involved residues L35, M37, L50 and F53 (44). The redocking experiments performed successfully, and the Glide software could correctly predict the binding mode in the cavity, i.e., reproducing the H-bond and stacking interactions involving F33 (Fig 2A). However, the inhibitor solvent-exposed region suffered a slight twist to interact with F33' side chain (on the other monomer). This change was somehow expected since the experiments were carried out with the homodimer and not with the tetramer that

stabilizes the ligand's conformation. For this reason, preparation protocols were assumed valid, proceeding next with the mutant structures analyzes.

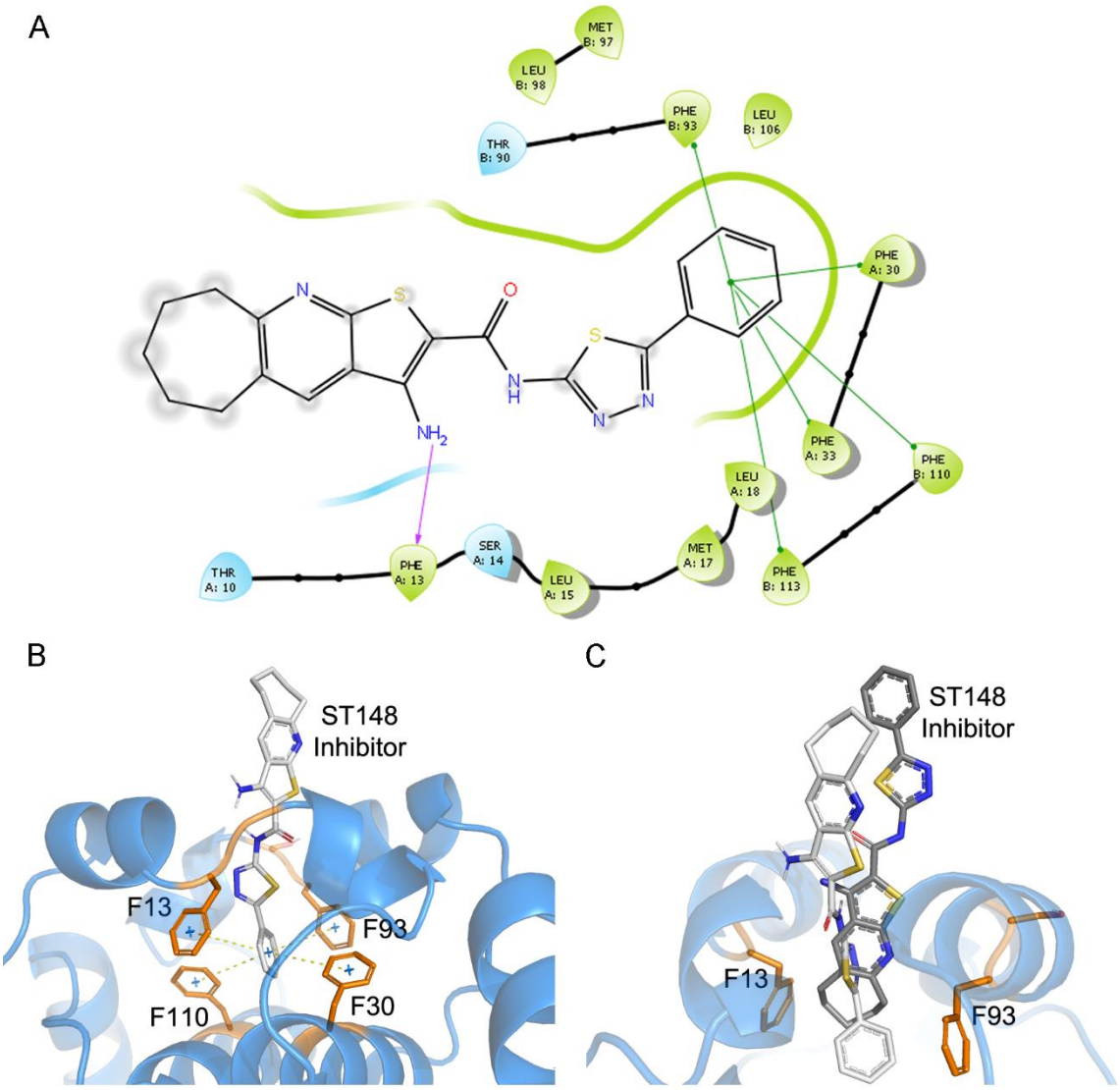



**FIG 2** Docking analysis performed with the C L50F mutant model and ST148 inhibitor. A and B) Predicted binding of ST148 inhibitor within the F50 mutant model, with formation of T-stacking interactions with Phenylalanine residues at the bottom of the cavity (green lines in A, dotted lines in B), while keeping the H-bond with F13 backbone (corresponding to F33 in 6VG5). C) Comparison of the experimentally predicted binding for the 6VG5 complex (dark grey) and the one obtained for the F50 mutant (light grey). L50F caused a 90º rotation of the inhibitor's position, leading to new interactions with F13, F93 (corresponding to F33 and F33'in 6VG5), F30 and F110 (corresponding to F50 and F50' in 6VG5) residues at the bottom of the cavity.

The L50F docking experiments showed a 90º turn of the ST148 inhibitor within the cavity (Fig 2C), triggering the formation of a new H bond with S34 in the middle of the pocket, and hydrophobic and stacking interactions with residues F33 and F50. These interactions seemed to further stabilize the inhibitor in the cavity, reducing the solvent-exposed area of the homodimer. As experimentally determined, the inhibitor induces a "kissing" interaction between two naive capsid dimers, resulting in defective uncoating of nucleocapsid (44). We speculated that if a tetramer carrying the L50F mutation somehow improved this "kissing" between the units, then the interaction between these domains should also increase. However, the entropy/enthalpy balance did not appear to support this possibility, and on the contrary, the ST148 inhibitor's affinity resulted slightly lower than that obtained in the experiments considering the wild structure: -6.935kcal/mol and -7.875kcal/mol, for F50 and L50, respectively.

On the other hand, residue T71 protrudes from a2 alpha-helix and is exposed to the C surface (Fig 1). It has not yet been described whether this position could be involved in C-C interaction when nucleocapsid formation. Nevertheless, when mutated for Alanine (as found in a DF case), changes in side-chain volume and polarity were barely evident over the protein surface. Therefore, it is suspected that any interaction as such should not be affected by this mutation. Finally, substitution R82I detected mainly amid DF cases as an iSNVs, would not impair a4-a4' interactions since its side-chain faced downward. However, this substitution meant losing a positive charge per monomer in a region where negatively charged RNA interacts. We hypothesize then that this mutation might slightly interfere with C-RNA interaction.

*Membrane precursor*

The immature precursor of M protein (prM) consists of an N-terminal peptide of 91 aa (pr peptide) which is cleaved by the cellular Furin during the virion maturation process, an ectodomain (residues 92-130) and a C-terminal membrane anchorage region (residues 131-166). The primary function of the pr peptide is to protect the immature virion against premature fusion with the host membrane when transported

through the cellular secretory pathway (47). The pr peptide secondary structure consists of seven β-strand, mostly antiparallel, as shown in Fig 3.

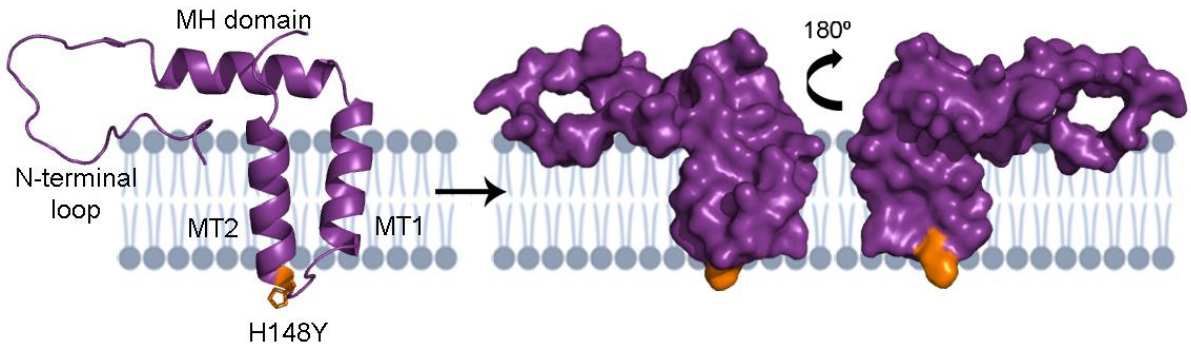



**FIG 3** Structure of DENV-2 pr peptide, residues 1-81. Cristal 3C5X was employed as a template for modelling. A) On the left, the cartoon diagram with the determined secondary β-strands. The surface diagram is represented in the middle and on the right. Mutations' locations and residues are denoted in orange. B) On the left, the surface diagram of the interacting area with protein E, with the charged residues involved denoted in red and blue. Mutations N7H and I39M are labelled. The opposite face, on the right, shows in grey the area concentrating the most common residues acting as epitopes for antibodies recognition. Mutation G15S is labelled.

Four different mutations were detected in this peptide, being all located within loops connecting beta-strands (Fig 3). According to previous studies, none of them were involved in contacting E protein, nor among the seven commonly recognized residues where prM antibodies mapped (47, 48). N7H was detected as an iSNV mainly amid DF cases. None of the amino acids involved in this substitution interacted with other residues by their side chains. However, R6 is a residue involved in prM-E interaction. The substitution of Asparagine for Histidine would not alter that interaction but would be contributing to an extra positive charge to the area. It is not clear if this could impair the close contact between prM and E protein. If such a case, it would be in line with the fact that most cases presenting this mutation developed a mild disease.

On the contrary, the remaining three mutations were detected mainly amid WS+SD cases. Residue G15, located within the bc loop (Fig 3A), was positioned on the opposite side of the pr-E interface and in the middle of a surface's valley. It colocalizes next to F1 and E18, two of the seven residues commonly detected by

antibodies. Thus, when mutated, as occurred at the intrahost level in three WS+SD, the Serine side-chain protrudes in this valley (Fig 3B), potentially creating a new epitope for antibody recognition. Substitution D29N was detected in 10 WS+SD and just 2 DF cases (Supplementary Table S1). Residue D29, located within the cd loop (Fig 3A), interacts in a 3A polar contact with T71 in fg loop, presumably contributing to secondary structure stabilization. Mutation D29N did not disrupt this interaction. Both amino acids protruded from the surface (Fig 3A). Although it is not considered one of the primary residues interacting with antibodies targeting prM, it was pointed out in previous studies as a residue involved in linear epitopes inducing a humoral immune response in mice (49, 50). Considering that cases carrying this substitution were mainly WS+SD cases and primary cases with at least five days of symptoms, or secondary ones, it could be likely that it might have arisen due to intrahost humoral selective pressures. The last mutation detected on the pr peptide was the iSNV I39M, found in three WS+SD cases; curiously, three cases also carrying the previously mentioned mutation. Both amino acids are non-polar, protruded from the surface (Fig 3), and located proximally to E62 and D65 involved in pr-E interaction, making residue 39 less accessible for antibodies targeting. In any case, it did not resemble a mutation of impact within the pr peptide.

Finally, after pr cleavage, the M protein consists of an N-terminal loop (residues 92-111), an α-helical domain (residues 112-131) and two transmembrane domains

named MT1 and MT2 (16). Modelling of M protein was performed based on crystal 7BUD (Fig 4).

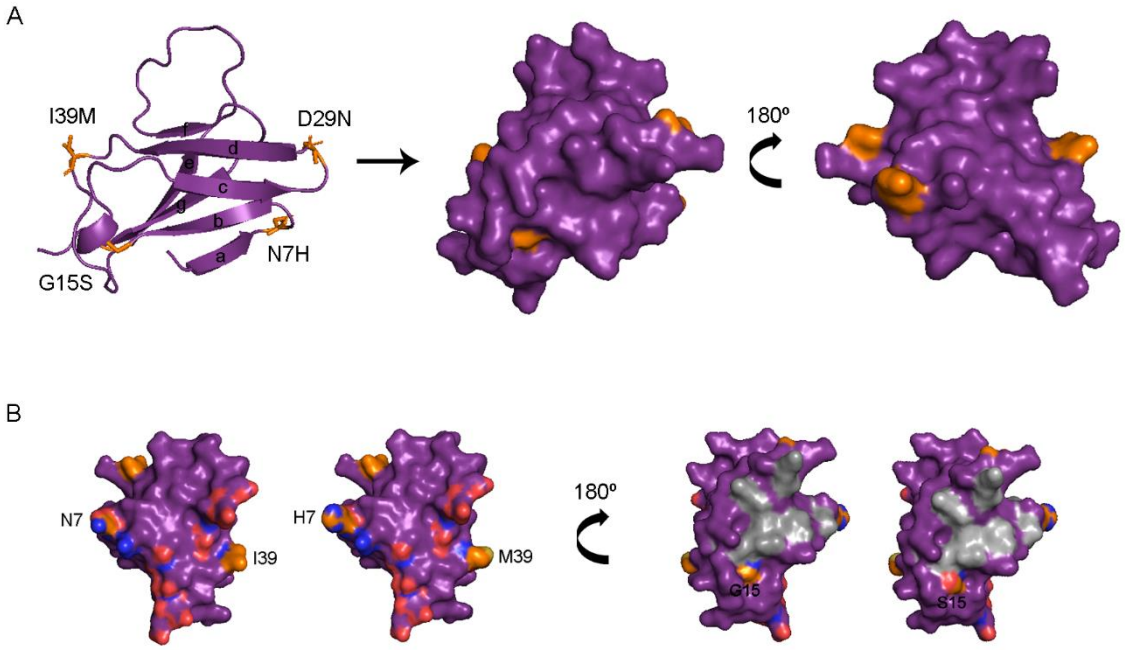



**FIG 4** Structure of DENV-2 M protein, residues 92-166. Cristal 7BUD was employed as a template for modelling. On the left, the cartoon diagram with the determined secondary alpha-helices. The surface diagram is represented in the middle and on the right. The location of residue 148 involved in substitution H148Y is denoted in orange. In grey, the phospholipid membrane where MT1 and MT2 are embedded.

A single iSNV detected in 5 WS+SD plus one DF case, H148Y, mapped within the loop connecting the two transmembrane a-helices (Fig 4). Although the amino acid substitution implied a substantial biochemical alteration, no structural alteration seemed to be caused. Besides, no function has yet been described for this loop, nor any relevant interaction. Due to its orientation, it could be hypothesized that it could at most interact with the hydrophobic face of the C protein, if not with other viral or host proteins, before virion assembly.

*Envelope*

Protein E is exposed at the surface of infectious mature DENV particles, as 90 homodimers lying flat against the surface (51), as schematized in Fig 5. It consists of

an ectodomain conformed by domains I, II and III, and a stem region (residues 395-449) connecting with the transmembrane anchor (residues 450-495). The N-terminal domain I is a structurally central beta-barrel formed by residues 1-52, 132-192 and 272-299. Domain II (residues 53-131 and 193-271), an elongated finger-like structure, is considered the fusion or dimerization domain, containing a hydrophobic fusion peptide (residues 98-110) involved in attaching the virus to the target cell membrane (52, 53). Domain III (residues 300-394) acts as the receptor-binding domain, primarily through its fg loop (residues 381-386), which forms a compact solvent-exposed bulge and has been demonstrated to be a determinant for viral entry into cells (53, 54). Besides, two conserved N-linked glycosylation sites are located at N67 and N153 (51). Residues representing important epitopes for antigen recognition have already been described in all three domains. Also, complex epitopes have been mapped to the inter-dimer interface (54-56). To determine whether the mutations described here caused any alteration over the protein's structure and/or function, an E-monomer model based on crystal 4UT6 was built as exposed in the previous section (Fig 5).

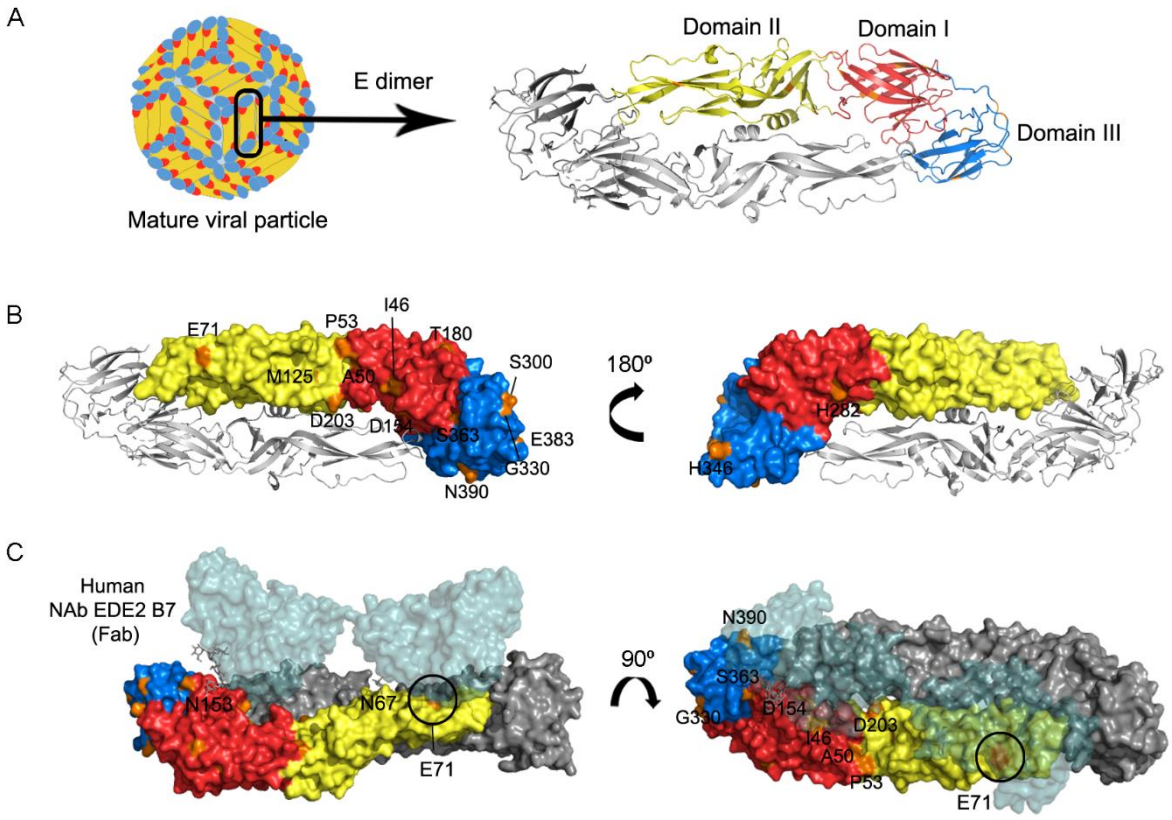



**FIG 5** DENV-2 E structure in its mature-virion conformation. A) E monomer, residues 1-395, was modelled based on crystal 4UT6. On the right, the ribbon diagram with the determined secondary structures. Domains I, II and III are shown in red, yellow and

blue, respectively. B) Surface diagram of the modelled monomer, dimerizing with the other monomer obtained from crystal 4UT6, shown as ribbon. Residues' location involved in substitutions is denoted in orange. On the left, the solvent-expose face, leaving the membrane-associated one on the right. C) Complex with Fab neutralizing antibody (Nab) EDE2 B7, co-crystallized with E dimer [54]. The E dimer in its surface scheme in side-view with the viral membrane-facing down (left) and seen from above (right). Monomers are colored as in B, while the Fab portion of Nab EDE B7 is represented in green. Glycosylated residues N67 and N153 are labelled on the left, with glycan residues shown as sticks. Residues involved in mutations under study and located within this dimer face are denoted, with E71 directly interacting with EDE2 B11 outlined in black.

Seventeen different substitutions were selected for analysis, and residues involved mapped into the model. Two of them, spanning residues M183 and T340 and located on Ho beta-sheet of domain I and d-e loop of domain III, respectively, were not highlighted in Fig 5 since they were not easily accessible on the protein surface. Together with residues T180, S300 and E383, exposed on the protein surface, these five residues were located on the dimer's lateral faces, i.e., not on the solvent-exposed side, nor the membrane-associated one. Substitutions T180I, M183I, T340I, and S300A, did not show to impair protein folding, while E383G caused a significant charge and volume alteration, upgrading flexibility of the surrounding area. Under these circumstances, it remains unclear whether these substitutions could be related to any interaction between the E dimers themselves. In such a case, the latter, detected at consensus level in a single SD case, would be expected to have the most significant impact. On the other side, substitutions H282Y and H346Y detected in a DF case and amid WS+SD cases, respectively, involved residues facing the membrane. They did not disturb secondary structures but represented a critical volume and charge disruption that could be beneficial since Tyrosine is a less polar residue, potentially favoring membrane interaction. Finally, the remaining substitutions involved residues located in the E dimer's solvent-exposed face (Fig 5C). Several studies had already correlated specific residues in the three domains with antibodies recognition, particularly those located at the inter-dimer interface or the DI-DII hinge on one subunit

and DIII on the other (54, 56, 57). Mutations P53L, E71A, D154N, G330D and N390S spanned residues previously implicated in diverse epitopes (Fig 5C) (56, 57). Particularly, E71A, D154N and G330D were found mainly among WS+SD cases, which belonged to secondary infections, or primary ones with at least four days of symptoms (Supplementary Table S1). Thus, they could be thought of as variants surpassing the humoral immune barriers. On the other hand, mutations I46M, A50T, M125I, D203N and S363A were located directly next to residues already proven to be targeted by antibodies, as K47 (55), K124, E126, E202, K204 (57), and D362 (56), among others. In this regard, it would be likely that these substitutions were at some point arisen due to the antibodies' pressures.

## Non-structural proteins

### *NS1*

DENV NS1 is a non-structural N-linked glycoprotein synthesized as a monomer that dimerizes after its post-translational modification in the ER lumen. Each dimer consists of three domains: a small beta-roll dimerization domain formed by two intertwined beta-hairpins (residues 1-30), a wing domain formed by a ab subdomain (residues 37-152) and a discontinuous connector (residues 31-36 and 153-179) that packs against the beta-roll and links the Wing to the central domain, and a beta-ladder domain (residues 180-352), a continuous b-sheet that extends along the dimer length, conformed by antiparallel beta-strands arranged like a ladder (Fig 6A) (58, 59). The beta-ladder defines a plane through the NS1 dimer, leaving the beta-roll and the Wing connector subdomain on the other face, creating a protrusion of hydrophobic character, denoted in Fig 6A with a dotted-line circle (58, 59). It has been proposed that through this area, NS1 interacts with the ER membrane and other viral proteins like NS4A and NS4B. Through these interactions, NS1 might organize the replication complex, becoming an essential element for replicating the viral genome (58). Besides locating in the viral replication complex, NS1 has also been found on the plasma membrane and the extracellular compartment. In the latter, it can be found as a soluble proteolipid particle with an open barrel hexameric shell associated with lipids in its

central channel. This NS1 hexameric form interacts with the complement-mediated immune system (58). Thus, to determine the potential effect of mutations here described, the NS1 model was constructed as a monomer subunit of the functional homodimer, based on crystal 4O6B (58). To notice, the bioinformatics approach here implemented allowed the modelling of a 24-residues disordered region of the wing domain that was unresolved in the reference structure (amino acids 107-130).

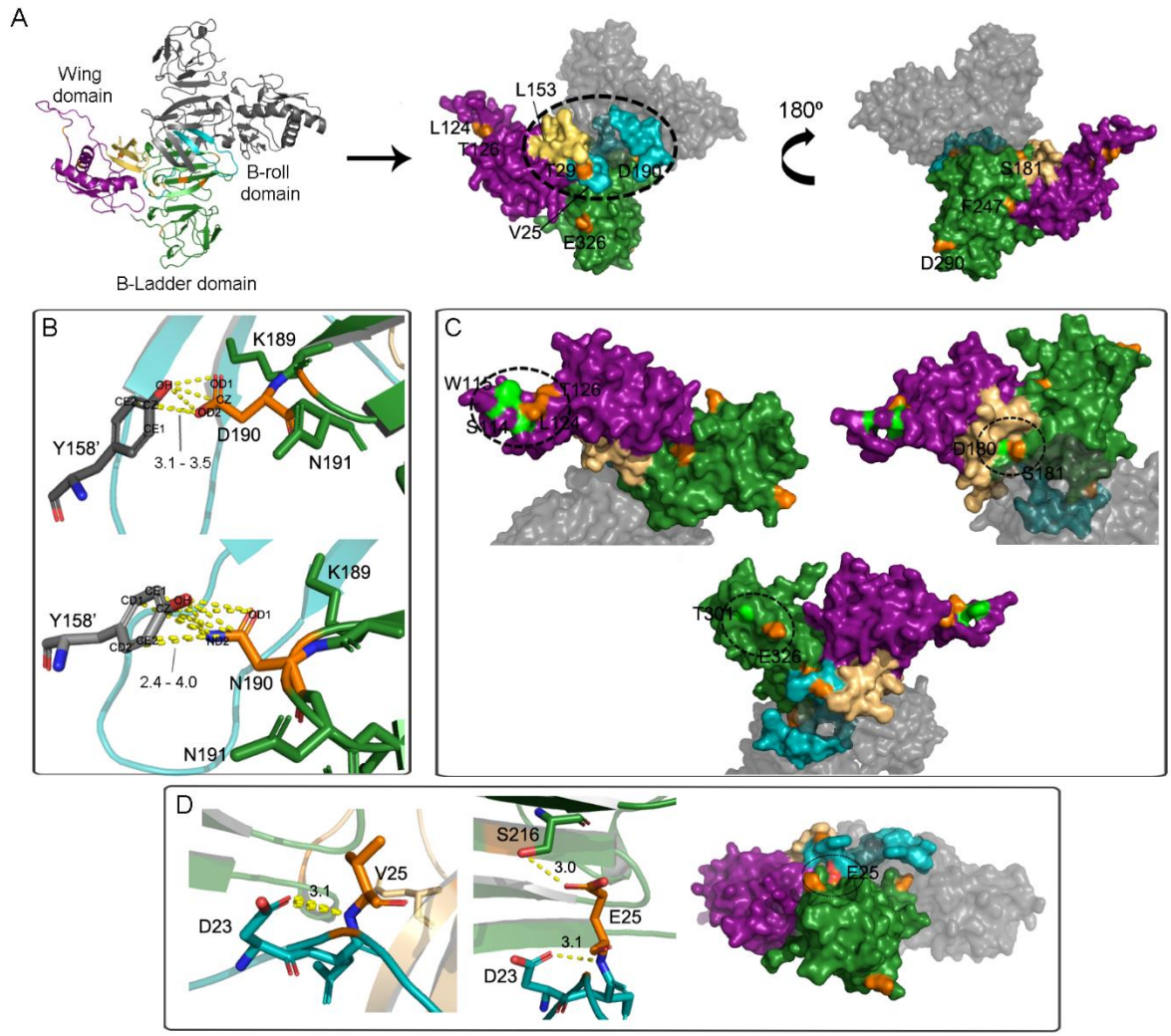



**FIG 6** Structure of DENV-2 NS1 protein, residues 1-352. Cristal 4O6B was employed as a template for monomer modelling. A) On the left, the cartoon diagram of the dimer. The b-roll (cyan), Wing (purple) and b-ladder (green) domains with their determined secondary structures. The Wing's connector subdomain is represented in light brown. On the right, both the dimer's faces are represented in the surface diagram, with the hydrophobic region circled with a dotted line. Residues involved in mutations are denoted in orange. B) Inter-dimer polar interactions between Y158' on one monomer and residues at position 190. C) Areas where specific residues (green) were previously

demonstrated to be involved in NS1-C interaction. Residues involving mutations under study (orange) mapped next to them. D) New polar interaction with S216 caused by substitution V25E.

Thirteen substitutions were detected for this protein in this study's samples. Of them, ten were exposed on the NS1 surface (Fig 6). The remaining three were located in residues Q47, I202, and M2012. They were internal and detected on a1 of the wing domain, and b3 and b4 of the beta-ladder domain, respectively. Substitutions Q47K, I202M and M212I were then considered conservative since their respective polar interactions with other residues stabilizing the secondary structure (Q47-S128, I202-H195 and M212-R257+Y258) were through the main backbone or just not disrupted when mutated.

Regarding the surface-exposed substitutions, two mutations were detected within the beta-roll domain: V25E and T29I. Residue T29 was located on the hydrophobic surface of the dimer (Fig 6). Mutating it for Isoleucine led to a reduction in the area's polarity, resulting possibly in a beneficial effect. Notably, this mutation was detected at the consensus level of an SD case. Conversely, mutation V25E would cause the introduction of a positively charged residue that also created a new polar interaction with S216 in b4 of the beta-ladder domain (Fig 6D). This interaction could likely decrease the loop's flexibility connecting b2 in the beta-roll with the connector subdomain. To notice, V25E iSNV was found mainly amid DF cases.

On the other hand, residues L124 and T126 carrying substitutions L124F and T126I were located in the Wing disordered and solvent-exposed loop. On one side, previous findings demonstrated that this loop was possibly engaged in NS1 interaction with viral structural proteins, showing a simultaneously abrogated interaction when residues S114 and W115 mutated to Alanine (17). Considering their close location to L124 and T126 (Fig 6C), it could be thought that the latter may also be involved in NS1-C/prM/E interaction. On the other side, linear epitopes have been mapped into this loop in the hexameric-secreted NS1 (58). Being mutations evading the immune response could be a reasonable explanation, mainly for T126I, detected in a SD case

with 10 days of symptoms. L124F was found at consensus level in three primary DF cases and as iSNV in seven WS+SD cases, mainly secondary infections.

Furthermore, substitution L153M did not cause any structural disruption since all this residue's interactions were via the amino acid main-chain. However, when L153 mutated for Alanine, it severely impaired viral replication (17). Functional studies would be needed in this case to determine if its mutation for Methionine also could have a similar phenotypic effect, but if so, it could be consistent with the fact that L153M was mainly found amid DF cases. Finally, five mutations were mapped to residues on the beta-ladder domain. Besides increasing the rigidity of the loop containing it, S181P, located like E326G, proximally to residues that, when mutated, abrogated the NS1-C interaction (Fig 6C) (17). Curiously, while substitution on S181 was found at consensus level in a single WS case, E326G was detected in 13 DF cases and just one WS case, which could be in line with the milder clinical outcome presented by the latter. Moreover, substitution D190N detected in 12 WS+SD cases, while only in two DF cases, showed slightly strengthening the contact area between the two monomers by creating new polar interactions between Y158' and N190 (Fig 6B). Regarding F247L, even though this substitution resulted in introducing a bulkier residue, it located within the "spaghetti loop" (residues 219-272), an unstructured region that could accommodate the F side chain by rotating other residues, slightly modifying the protein folding. This mutation was encountered both in DF and WS+SD cases and irrespective of the patients' immune status. It remains unknown whether this residue could interact with other proteins, but the mutation looked merely conservative. Finally, D290N mapped within a conserved tip region (residues 278-352) which has also been implicated in antibodies recognition (58). This residue protrudes from the surface, turning it into an accessible epitope (Fig 6A). By mutating from Aspartate to Asparagine, the net charge on this residue's surface gets reversed. This substitution was detected in three WS+SD cases, belonging to one primary case with six days of symptoms and two secondary cases. Thus, this could be in line with the chance of this variant representing a circumventing one to the humoral immune response.

*NS3*

NS3 protein is composed of an N-terminal domain (residues 1-168) with a trypsin-like serine protease activity responsible for several polyprotein cleavages with the release of viral proteins and a C-terminal domain (residues 180-618) with enzymatic activities of RNA triphosphatase (RTPase), RNA helicase and nucleoside 5' triphosphatase (NTPase) (3). An 11-residue linker connects both domains, whose flexibility is essential for the domains' different conformations and activities, profoundly affecting the replication efficiency (60). Homology modelling of the complete NS3 was performed with crystal 5YW1 as a template. Although it corresponded to a crystal of DENV-4, the structure presented better alignment scores at PDB advanced search and BLASTp. Seven relevant amino acid substitutions were detected mainly amid DF cases, and the residues involved were mapped into the model (Fig 7A). Notably, a single mutation was detected within the protease domain, while the remaining six were located one in the linker and five in the helicase domain. The former mapped to the protease's N-terminal unstructured tail without causing any relevant structural disorder. Although the substitution G14E resulted in the shift of the net charge in the surface area due to the carboxyl group in the Glutamate side-chain, it did not affect any of the protease-related motifs nor the areas involved in cofactor NS2B interaction (Fig 7B-C).

Whether this mutation could somehow modify any other interaction with other viral or host proteins remains still unknown.

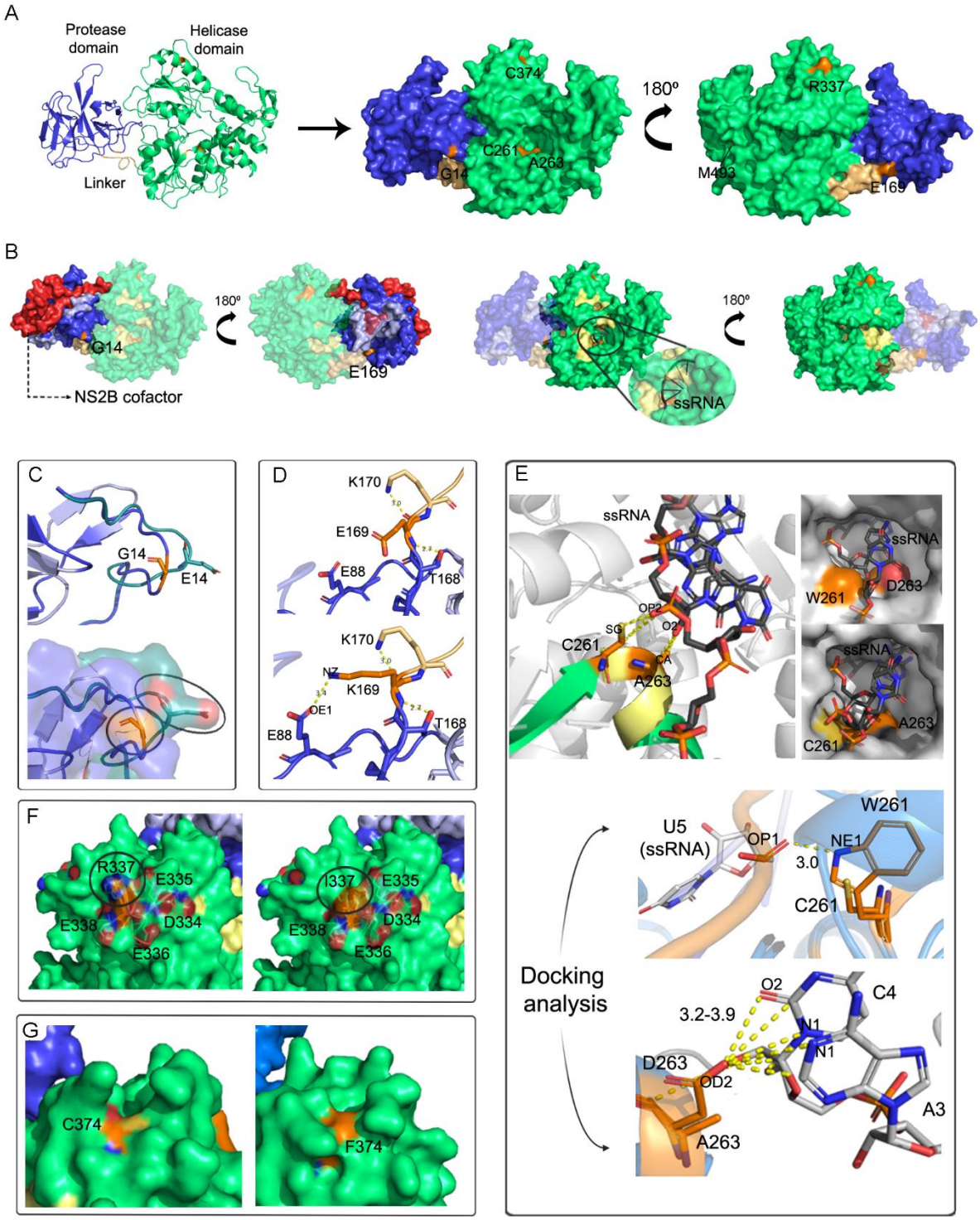



**FIG 7** DENV-2 NS3 protein structure (residues 1-618) modelled based on crystal 5YW1. A) On the left, the cartoon diagram with the N-terminal protease domain in blue and the C-terminal helicase domain in green. The linker is represented in light brown. On the right, its two faces are represented in a surface diagram. Residues involved in

mutations are denoted in orange. B) On the left, protease motifs denoted in light blue, with the catalytic triad comprising residues H51, D75 and S135 highlighted in purple. Also, the NS2B cofactor obtained from crystal 2FOM is shown in red. On the right, the helicase superfamily motifs are denoted in pale yellow. Zoomed in, the contact region with ssRNA, ligand obtained from 2JLU. C) Loop shifting and surface net charge disruption caused by substitution G14E. D) New polar bond set up with protease residue E88 as a consequence of E169L substitution. E) C261 and A263 interactions with ssRNA (obtained from 2JLU crystal). When mutated to W261 and D263, these residues' side-chains narrowed the channel for ssRNA passage. However, docking analyses showed a possible rearrangement of these mutations' lateral residues within the channel that, in fact, seemed to strengthen the interaction with the ssRNA. Electrostatic interactions between W262 and the phosphate group of Uracil 5, and between D263 and the N1 of the Adenine 3 and N1 plus O2 of Cysteine 4 are shown in yellow. F) DEERE charge patch on the helicase surface spanning residues 334-338. Substitution R337I led to the reduction of the electric charge on this region's surface. G) Overall surface rearrangement caused by C374F.

NS2B3 protease complex cleaves the viral polyprotein into several different points, releasing the viral proteins C (from its transmembrane anchor), NS2B, NS3, NS4A, NS4B, and NS5 (2), while host furin and signalase proteases do collaborate with the processing of the remaining viral proteins (2) (Supplementary Figure S6). Previous experimental analyses have demonstrated that the NS2B3 highly conserved residual preferences at the cleavage sites included dibasic residues at P1 and P2 positions, basic or aliphatic residues at P3 and P4 positions (upstream the cleavage site), and a small or polar residue at P1', a weak acidic residue at P2', a small and polar one in P3' and a weak and small one in P4' (downstream the cleavage site) (61) (Supplementary Figure S6). Thus, substitutions over these particular positions could alter the enzyme kinetics and, thus, the polyprotein cleavage efficiency (61, 62). Additionally, recent evidence showed that inhibiting one particular self-processing event leads to trans-dominant inhibition of RNA replication (63). Therefore, it was checked whether any of the substitutions under study were located in any of the targeting cleavage site of the NS2B3 protease. Three substitutions spanning the P4 or

P4’ position at the C-C transmembrane anchor (V104M), NS2A-NS2B (S215N) or NS4B-NS5 (N245S) cleavage sites were detected mainly amid WS+SD cases, excepting the latter one, present at consensus level in a single DF case (Supplementary Figure S6). However, none of them were expected to truly disturb the cleavage enzymatic activity: residue 104 in the C protein has been previously described as a conserved Methionine among the four DENV serotypes (62), and both Asparagine and Serine have been described at position P4 of the cleavage sites, with a similar enzyme specificity (61). It is important to mention that the NS2B3 protease complex also cleaves host proteins like STING, among others, a mechanism through the one the virus succeeds to inhibit cellular IFN-I production, and thus, dodges one of the host antiviral responses (64). Nevertheless, how this phenomenon may correlate with disease severity within this cohort escapes the reach of this study. It is important to mention that the NS2B3 protease complex also cleaves host proteins like STING, among others, a mechanism through the one the virus succeeds to inhibit cellular IFN-I production, and thus, dodges one of the host antiviral responses (64). Nevertheless, how this phenomenon may correlate with disease severity within this cohort escapes the reach of this study.

On the other side, located in the linker's first residue, substitution E169K created a polar contact between the Lysine and E88 (Fig 7D). Since this new interaction could stabilize a conformation where ATP binding would be favored, the enzyme activity and, thus, RNA replication are not suspected to be disturbed. As well, a salt bridge interaction has already been described for DENV-4 between E169 and K88, residues commonly encountered in these positions (as in GenBank AY776330) (60).

Concerning the five substitutions involving residues in the helicase domain, C261W and A263D compromise two positions in motif Ic (residues 261-265), a region of the helicase active site interacting with the single-stranded RNA (ssRNA) once the NS3 unwinds the double-stranded RNA viral intermediate (3). Particularly, C261 interacted with the oxygen O3' and the phosphate P of the phosphodiester bond between bases C4 and U5 of ssRNA ligand (PDB 2JLU) and with the OP2 oxygen in the U5 phosphate group (Fig 7E). Also, A263 interacted with oxygen O2' and carbons C1'and C2' of the pentose ring in C4 (Fig 7E). Thus, it became clear that these interactions contributed to stabilizing the emerging ssRNA. When mutated to

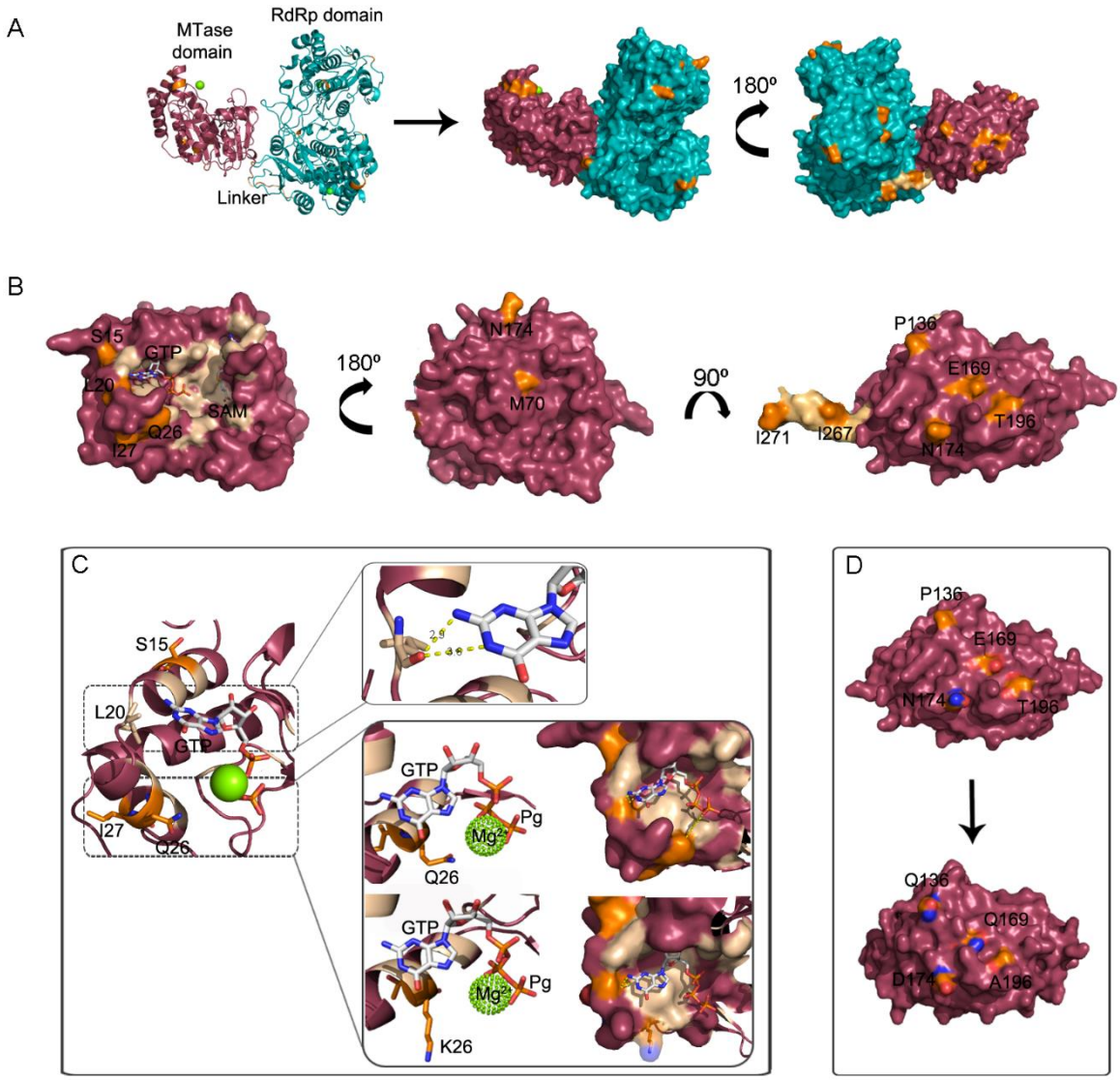
Tryptophan and Aspartate, respectively, the side-chain of these amino acids reduced the space to accommodate the ssRNA (Fig 7E), and therefore, it can be hypothesized that the enzyme efficiency may be somehow limited. Plus, D263 exposed its side-chain negative charge on the channel' surface (Fig 7E). Since this substitution was detected in samples also carrying C261W, it could be thought that it might counteract the latter's effect and so be selected. However, this assumption is not an obvious observation, considering the above mentioned. Therefore, to confirm the previous hypothesis, redocking and cross-docking experiments were performed with structures 2JLU and 5WY1 against the ssRNA fragment (crystallized in complex with structure 2JLU), using Glide as a pose predictor. However, this software was unable to predict any pose similar to the experimentally observed. Despite occurring in many different cellular processes, analyzing the protein-RNA interaction is still a challenge due to freedom degrees. For this reason, the redocking experiments were carried out using the HDOCK server (http://hdock.phys.hust.edu.cn/) (36), one of the commonly described servers in the literature for predicting this type of interaction. The entry structures were the same as those prepared by Maestro suite and used in the Glide experiments. The HDOCK predicted an excellent geometry for RNA molecules in 2JLU and 5WY1 protein structures with a template-based methodology, allowing the subsequent analysis with the mutant models, employing the same parameters previously described. Docking studies indicated that both mutations could increase the binding affinity between the RNA and the protein cavity (Fig 7E). This potentially strengthened interaction could result in two possible outcomes: i) an increased enzyme efficiency through a stronger interaction between the receptor and the RNA, facilitating the RNA strings' opening by the helicase, or ii) a reduced enzymatic ability, since higher RNA stability could lead to a loss of efficiency in reading and opening the strings. However, further studies would be needed to confirm any of these hypotheses.

On the other hand, substitution R337I involved a residue of the "DEERE" charged amino acids patch (334-338). It has been demonstrated before that these charged residues mutating to non-polar Alanine reduced the ATPase and helicase activities of NS3 (65). In this case, the substitution for Isoleucine also meant the decrease of the surface net charge (Fig 7F). It could be suspected then that this mutation may affect enzymatic activity as well. Regarding substitution C374F, this residue was located in a a-helix, exposing its side-chain to the surface, but did not

make up any of the motifs involved in the protein active sites. Even though the residue's volume would be greater in the mutated protein, no known protein-protein interaction covering this residue has been described by now. It is important to highlight, in any case, that mutations altering conserved, non-enzymatic surface residues, as R376A, have been shown to reduce viral fitness (63). Thus, further studies would be needed to elucidate if this mutation causes any non-conservative effect. Finally, substitution M493I resulted in a conservative one, without causing significant physicochemical disturbances in the alpha-helix where it mapped or its contiguous region. Curiously, the seven NS3 mutations here analyzed were mainly detected in DF cases. Therefore, considering all the above discussed, if any of these variants caused, in fact, any detrimental effect for viral fitness, it could be in line with the milder clinical outcome evidenced in these patients.

*NS5*

DENV-2 NS5 is the largest and the most conserved of the viral proteins. It is composed of an N-terminal domain (residues 1-263) with methyltransferase activity (MTase) and a C-terminal domain (residues 273-900) with RNA dependent RNA polymerase (RdRp) activity. A 10-amino acid linker connects both domains and acts as a critical determinant of the global conformation and activity of NS5 (5). This protein's homology modelling was carried on with crystal 5ZQK as a template, with the resulting model plotted in Fig 8A.



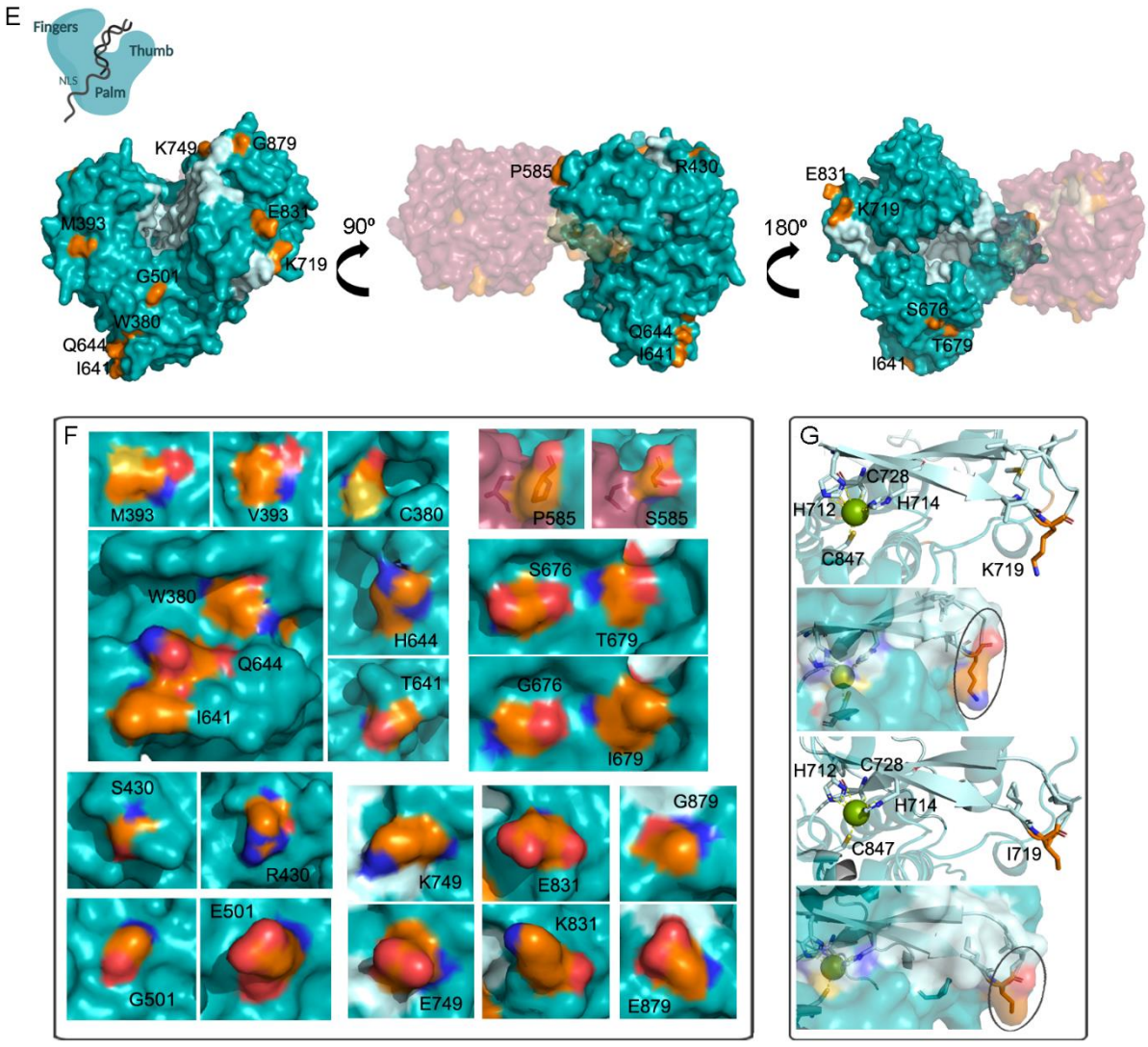



**FIG 8** 3D structure of DENV-2 NS5 protein (residues 1-883). A) On the left, the cartoon diagram with the N-terminal MTase domain in purple and the C-terminal RdRp domain in cyan. The linker is represented in light brown. On the right, its two faces are represented in a surface diagram. Residues involved in mutations are denoted in orange. B) MTase domain with catalytic motifs denoted in light brown. In the third surface representation from right to left, the linker subdomain is shown in light orange. Residues involved in mutations are indicated and highlighted in orange. C) Effect of mutations in L20 and Q26 on interaction with GTP, one of the essential substrates for RNA methylation. GTP and Mg2+ ligands were obtained from crystal 4VOR. D) Electrostatic-charge shift caused by mutations P136, E169, N174, and T196, whose amino acids' side chains were facing the protein surface. The red color (negative potential) represents an excess of negative charges near the surface due to oxygen's presence, while the red (positive potential) represents a positively charged surface,

usually in line with the nitrogen's presence. E) Surface representation of the RdRp domain in its classical right-hand architecture (left), with the catalytic motifs highlighted in light blue. Residues involved in mutations are indicated and denoted in orange. F) Disturbances in the electrostatic charge pattern on RdRp's surface caused several of the mutations described here. G) Localization and effect of mutation K719I within the catalytic E motif containing the Zinc binding pocket.

Besides the MTase activity, the N-terminal domain also performs as a guanylyltransferase. Both activities are fundamental for the RNA capping, whereby the viral RNA gains stability, binds to ribosomes for an efficient translation, and gets protection against degradation by the 5' to 3' exoribonucleases and the host cell immune sensors (66). Critical regions implicated in these activities include the MTase conserved active site spanning residues K61, D146, K181 and E217, the SAM binding pocket, where the S-adenosylmethionine (SAM) binds and acts as a methyl donor, and GTP and RNA binding sites (67). They were highlighted in the model (Fig 7B) and visually inspected if any of the ten mutations mapping into the MTase domain was involved in these motifs. S15R, L20M, Q26K and I27T compromised residues located in A1 alpha-helix, the A1-A2 loop, and A2 alpha-helix, respectively; a region committed to GTP binding and RNA capping (68, 69). Although L20 directly interacted with the Guanine's nitrogen stabilizing the GTP molecule, it did through its main-chain oxygen and not via the side-chain (Fig 8C). Thus, the substitution for Methionine, a residue with similar chemical properties, proved to be irrelevant. On the contrary, S15 and I27 seemed located on their respective alpha-helices' outer side, with their side chains pointing towards the active site's opposite side. Substitutions for R15 and T27 did not disturb secondary structures nor intrachain stabilizing contacts. Thus, they were considered conservative mutations. Similarly, Q26K was also considered a conservative substitution because even though Glutamine's side-chain pointed directly to the Mg2+ ion and the GTP's gamma phosphate, no clear interaction among them was detected since their distance was higher than 5.5A. When mutated to K26, the rotamers analysis showed that Lysine's side-chain oriented to the opposite side, turning it less likely to interact with the GTP (Fig 8C). Likewise, F65 showed to be an inner residue located in the A4 alpha-helix, not involved in any active site, while M70

was on the loop next to this helix but exposed to the protein surface, on the opposite side of the active sites. They interacted with each other via their main-chains, polar contacts that remained unaltered even when mutating F65S or M70L. Even though in the particular case of F65S the substitution meant a residue's stereochemistry and polarity shifts, no apparent reasons were demonstrating that they could be non-conservative mutations. The remaining four substitutions detected in the MTase domain committed the residues P136, E169, N174, and T196, whose side-chains were exposed on the protein surface. Curiously, their spatial proximity grouped them as within a patch (Fig 8B). P136 and N174 were located in two different loops, without presenting any polar contact with other residues, and so it remained in mutations P136Q and N174D, except that Q136 created a 3.4A polar bond via its amide group with P137 main-chain oxygen. Conversely, E169 and T196 were located in aD, and aE alpha-helices, respectively (68), and thus presented several polar and H-bond contacts with proximal residues stabilizing the secondary structures. Substitutions E169Q and T196A did not significantly disturb those bonds, and consequently, neither the secondary structures. What was striking about these four mutations is that they altered the electrostatic charge in this area, potentially modifying a protein-protein interaction (Fig 8D). Nevertheless, there is no clear relationship between the whole patch and the patient's clinical outcome or immune response (Supplementary Table S1). In general, none of these ten mutations assessed above showed to be able to impair the MTase enzymatic activities.

The linker domain, also known as the "inter-domain region" spanning residues 263-272, connects the MTase and RdRp catalytic domains, allowing them to adopt different conformations relative to each other upon binding to RNA, NS3 or host proteins. Even though its sequence is not strictly conserved, its folding is, guaranteeing the required protein flexibility (70, 71). Indeed, in a mutagenesis study of the residue I265 it was demonstrated that when mutated to Proline, the imposed rigidity abolished virus replication, while when mutated to Glycine, the increased flexibility slightly attenuated it (72). Substitutions I267T and I271T were found in an SD and WS case, respectively. However, they did not show to cause any impairment in the domain folding since their stereochemistry are similar, and the introduction of the –OH group only created polar contacts with solvent.

The RdRp domain revealed the canonical right-hand architecture with the finger (residues 273-315, 416-496 and 543-600), palm (residues 497-542 and 601-705) and thumb (residues 706-900) subdomains. The conserved enzymatic motifs A-G within them were highlighted in figure 8E. Motifs A, B, C, and D, are located within the palm domain and contribute to the cation-binding site, in the sliding of the RNA in the RdRp tunnel, comprise the GDD catalytic triad, and help to release a PPi after the NTP binding, respectively. Motif E is located within the thumb subdomain and houses the structural Zinc cation, while motif F, located on the fingers, helps stabilize the nascent base pair. As well, a priming loop spanning the residues 786-809 protruding from the thumb subdomain catalyzes the polymerization "de novo", serving as a platform for the polymerization activity and regulates the RNA access and exit into the active site (reviewed in 71). Besides the catalytic subdomains, an N-terminal region of the RdRp domain has been identified as an NLS region (nuclear localization signal, spanning residues 316-415), involved in NS3 and beta-importin interaction, among others. DENV-2 NS5 has been frequently detected in the cellular nucleus and ultimately linked to viral pathogenesis (73).

Fifteen substitutions mapping the RdRp domain were selected in this study as relevant for analysis (Fig 8E). Three of them, T377A, W380C and M393A, involved residues located within the NLS subdomain, in a6 alpha-helix the former, and a loop connecting a6 and a7, the latter one (73). None of them caused any disturbance in the secondary structures nor the intrachain stabilizing polar interactions. Thus, they were conceived as conservatives. However, these substitutions were detected exclusively in WS+SD cases, and W380 and M393 were exposed on the protein surface. In line with the aforementioned, further studies would be needed to trace potential relevant protein-protein interactions involving these residues. The remaining 12 mutations were found on the functional RdRp's subdomains. While only two committed residues located in the fingers' subdomain (S430R on a9 and P585S in the b2-b3 loop), six were mapped into the palm subdomain: G501E in the a11-a12 loop, E616G in a16, I641T and Q644H in a18, S676G in a19, and T679I in a20 (73). None of them localized within catalytic motifs or relevant known structures like the priming loop. Moreover, they were all found within loops and alpha-helices secondary structures, the ones that naturally present greater flexibility than beta-strands when it comes to better accommodate the presence of different amino acids, resulting in greater tolerance to mutagenesis. In

fact, none of them achieved to disturb significantly any of the corresponding secondary structures, nor the polar contacts within residues that contributed to their proper stabilization. Remarkably, however, excepting E616G spanning an internal residue, these mutations were exposed on the RdRp surface and brought about a shift in the exposed electrostatic charge (Fig 8F). Notably, P585 was located at 3.7A of S260 side-chain oxygen in the Mtase subdomain without interacting with each other. Its substitution for S585 did not modify this scenario since, in the current model, the serine side-chain turned towards the opposite side without diminishing the distance for possible contact via hydrogen bond (Fig 8F).

Finally, four substitutions were identified within the thumb subdomain and also located in loops or alpha-helices: K719I in b6-b7 loop, K749E in a22, E831K in a24-a25 loop and G878E in the C-terminal loop (73). To highlight, K719I was found within the E catalytic motif; however, it was distant from the Zinc binding pocket, plus its side-chain faced out to the surface (Fig 8G). Regarding the latter, also all these mutations were mapped over the protein's surface and entailed disturbances in the electrostatic charges exposed (Fig 8F).

Overall, NS5 mutations assessed in this study did not show to alter the protein's catalytic activities. Still, on the contrary, it could be suspected that the perturbations, if caused indeed, might be related to protein-protein interactions. Several contact points have been described between NS5 and NS3, the SLA in 3'UTR, host b-importin (74, 75), but none of them shows to be compromised by these mutations. However, NS5 interacts with many other host proteins related to spindle assembly, splicing, chromatin modifications, and immunomodulation, among others (7, 76). However, it remains unknown how they do it. Consequently, these mutations' role should not be dismissed, even though they might seem neutral to the efficient NS5 functioning.

*NS2 and NS4*

DENV NS2A-B and NS4A-B are the smallest non-structural viral proteins that started to draw attention in the last years due to the emerging evidence of their essential roles in viral replication and immunomodulation (26-29). However, as both are inserted in the endoplasmic reticulum (ER) membrane (26-29), no one was able to

properly model them in their entirety yet. Different strategies have been proposed to solve their topologies by employing biochemical assays and/or nuclear magnetic resonance, which was crucial to obtain pieces of their structure, ultimately puzzling them together into the most likely topology (Fig 9). Consequently, it becomes evident that no available models nor crystals could serve as a starting point for any classical homology modelling strategy. Thus, in this work we employed fold recognition and de novo strategies to build models that would then allow us to assess the effect of these proteins' mutations found in the patients of this study.

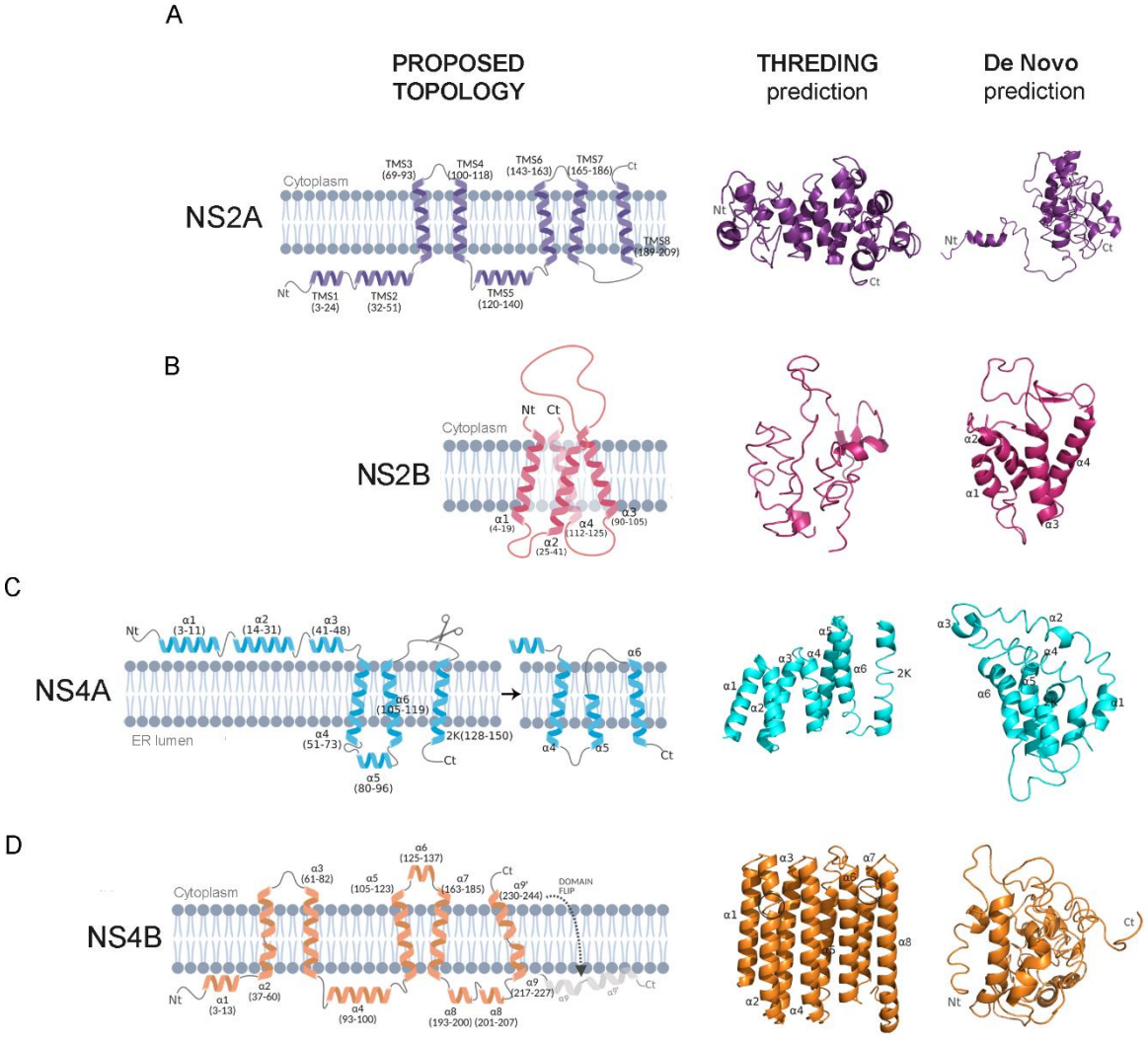



**FIG 9** Topologies proposed for membrane-associated proteins NS2A and NS4A-B, and folding recognition and de novo modelling predictions. From left to right, the proposed topologies and the models obtained with threading approach in I-TASSER (middle) or a *de novo* approach with DMPfold (right) for proteins NS2A (A), NS2B (B), NS4A (C) and NS4B (D). The ER membrane where these proteins are embedded is

colored in grey. TMS: transmembrane segment; Nt: N-terminal segment; Ct: C-terminal segment.

Several structural differences were observed in topologies predicted by I-TASSER and DMP-fold servers, up to 15Â in some cases. In general, I-TASSER was more accurate than DMP-fold, resulting in models more comparable to the theoretical topologies. For NS2A, the model constructed by I-TASSER was composed of helices and short helices, similar to the proposed one by Xie and colleagues (26). However, these structures were interspersed by non-folding parts, resulting in many residues in unfavorable positions, i.e., only 50% of the predicted residues were in favorable regions in Ramachandran plots, while for a good structure, at least 90% are expected. A similar situation was observed for NS4A: the model obtained could be interpreted using the u-shaped topology (28), but only 58% of residues were in favorable regions. The best prediction was obtained for the NS4B model, where 83% of residues settled down on promising regions. However, the topology predicted by I-TASSER resulted considerably differently from the proposed one by Li and collaborators (29). Thereby, despite several attempts, even considering the existing topologies' coordinates as input information for modelling, the built structures did not fulfil the quality parameters required for an acceptable model. To model protein membrane-embedded topologies is still a significant challenge in the area. Software widely recognized in literature and commonly used for protein folding still find it quite challenging to solve this problem. It is necessary to consider the membrane in studies of model refinement through MD in such cases. These studies are computationally expensive because of the number of thermodynamics features to view. They need enough time to observe the folding in the membrane and force fields correctly parameterized for the problem. Thus, considering that they were not guaranteeing any analysis's accuracy, mutations were merely judged considering the topologies proposed and the information gathered for each protein' region.

NS2A is a 218 amino acids protein, for which a biochemical analysis suggested a membrane topology with two N-terminal transmembrane segments (TMS1 and 2) located in the ER lumen, five real TMS (TMS 3-4, 6-8), an additional TMS located in the ER lumen with no membrane-associated activity (TMS5) and a C-terminal region

(residues 210-218) in the cytosol (Fig 9A) (26). The N-terminal segments have been associated with DENV cytopathogenesis, while the C-terminal region was shown to be involved in the virion's assembly and secretion. This protein also collaborates in viral RNA synthesis, colocalizing with the double-stranded viral RNA (dsRNA) and interacting with structures in the 3'UTR within the replication complex, and possibly with NS3 and NS5. On the other hand, it has been shown that NS2A acts synergistically with NS4B to inhibit the antiviral cellular response (4, 77). Based on the proposed topology, mutations under study spanning residues T29, A37, F40, T63, T115, A151, S153, Q186, A189 and S215, involved TMS2, 4, 6, 7 and 8. Substitution T29A turned out to be one of the substitutions reported by Wu and colleagues as related to a reduction in viral yield in an in vitro mutagenesis study (78). This mutation was detected at consensus level in two DF and as iSNV in two SD cases. Thus, its relation to DENV clinical outcome remains unclear. Segments 25-41 and 103-183 have been shown to possess both a very low net positive charge and, consequently, can rupture lipid membranes (79). Mutations A37T, F40L, T63A, T115I, A151P and S153L did not seem to disturb this feature since they involved substitutions to non-charged or non-polar amino acids. Finally, mutations T115I and A189T/S stood out because they mapped within TMS4 and TMS8, respectively, which showed essential roles in RNA replication and virus assembly (80), plus they were exclusively found in WS+SD cases. In any case, functional studies would be needed to determine whether they could be correlated with DENV severe pathogenesis.

DENV NS2B is a 130 amino acids protein predicted to contain four relatively short transmembrane alpha-helices inserted in the ER membrane, leaving a hydrophilic segment of 40 residues exposed in the cytosol (residues 45-89), which acts as a cofactor for NS3 protease domain, allowing the correct folding, location and activity of viral serine protease (27). The NS2B3 complex also participates in the host's immunomodulation, inhibiting the type 1 interferon (INF) response and stimulating the apoptotic pathway in endothelial cells (81). On the other hand, it is believed that NS2B also plays a role in the replication complex, since its co-localization with dsRNA has been observed, and also as viroporin, which suggests that it would facilitate the cytopathic effect induced by DENV and at the same time, viral assembly and secretion (82, 83). It is worth mentioning that no mutation was selected as relevant for analysis in this gene. Indeed, NS2B was demonstrated in the preceding work as the less

variable gene irrespective of the clinical category, with just a few conservative amino acid substitutions or others at considerable low intrahost frequency (19). Hence, it could be considered as a potential target for antivirals design.

NS4A is a highly hydrophobic protein of 150 aa, shown to be composed of three amphipathic membrane-interacting alpha-helices at the N-terminal segment (a1-a3), and three highly hydrophobic transmembrane alpha-helices (a4-a6), that although embedded in the ER membrane, might undergo conformational changes upon maturation, i.e., after NS2B3 cleavages the 23-aa segment known as 2K (Fig 9C) (4, 28). The N-terminal region is located in the cell cytoplasm and plays a critical role in changing the ER membrane curvature, facilitating its invagination for later release of the immature virion into the ER lumen. This curvature also allows the assembly of the replication complex. It has been determined that mutations that alter the aforementioned amphipathic character abolish viral replication in vitro (28, 84, 85). The C-terminal transmembrane domain is involved in the oligomerization of NS4A itself, which occurs before the induction of curvature, and also is essential for membrane remodeling. In turn, through this region, NS4A interacts with NS4B, which is believed to be the key to modulating the transition from forming the vesicle pockets to the viral replication complex, and host proteins like Reticulon 3.1 and Vimentin, both implicated in this structural remodeling. Mutations within this area, mainly in a4, proved to impair viral replication (85, 86). On the other side, NS4A has been shown to regulate the host immune response, indirectly preventing interferon induction (87). Four significant substitutions were selected in this study for further analysis. According to the proposed topology and previous solvent exposure assays (28), Y41H, T42N and S46N would be located within a3 amphipathic alpha-helix, with the former on the hydrophobic face and the latter on the hydrophilic one. While substitutions on position 42 and 46 were not expected to alter the area's polarity, Y41H involved an exchange to a residue with a positive net charge in its side-chain. Previous evidence showed that mutations Y41A and Y41F disrupted the interaction between NS1 and the NS4A-2K-NS4B precursor, potentially affecting the formation or function of the DENV-2 replication complex. Notably, Y41A resulted not viable while Y41F impaired viral fitness and decreased the infectious virus production (88). It is not clear, though, whether Y41H, detected widely at consensus level amid DF cases, but as iSNVs in WS+SD ones, could cause such a drastic phenotype. On the other side, A63T also found mainly at consensus level in DF

but at iSNVs in WS+SD cases too, would be located in a4, the region that interacts with NS4B. Furthermore, it seemed implicated in a small-XXX-small motif formed by residues A62-G66 (being X any residue), frequently linked to transmembrane domain interactions (28). If such a case, the substitution for Threonine here detected, could somehow disturb the NS4A-NS4B interaction, ultimately affecting viral fitness, which could be in line with its relevant presence amid DF cases. Functional analyses would be needed to prove this hypothesis.

Lastly, NS4B is a glycoprotein of 248 aa, predicted to contain 11 alpha-helices (a1–9, a8′, and a9′), being five of them true transmembrane domains (a2, a3, a5, a7, and a9–9′; Fig 9D). Helices a9–8′ and a9–9′ form a single kinked helix separated by a helix break (29). Residues 125-162 form a long cytoplasmic loop comprising a6 and two β strands, through the one it interacts with NS3 (89). The N-terminal first 30 residues, plus residues 142–160 of the long cytoplasmic loop, are dynamic and solvent-exposed in the ER lumen and cytoplasm, respectively. The region spanning the residues 84-146 directly binds with NS4A. The C-terminal a9–9′ plus the tail region (residues 217-248) undergo conformational changes and flip from the ER lumen to the cytoplasm, where it interacts with NS5 (29). Besides participating in viral replication, this protein plays a crucial role in regulating the host's immune response by inhibiting the INF pathway, suppressing both the protein unfolding response and the formation of stress granules in response to viral infection, and the interfering RNA (RNAi) pathway (90, 91). Thirteen mutations found in patients of this cohort were considered relevant for analysis. Based on the predicted topology, only three of them mapped within defined alpha-helices: E7K in a1, T45A in a2 and L75S in a3, all mainly detected as iSNV amid DF cases. Regarding the former, since a1 is a solvent-exposed region, this substitution could probably become relevant if the residue were involved in any protein-protein interaction. In line with this rationale, eight mutations were located in the a1-a2 loop: L13F, F15L, R16G, T19A, T20I, E22Q, S23P and R33C. Thus, as a disordered and solvent-exposed region, this region would be expected to be more tolerable to mutagenesis. Finally, T216A and N245S involved residues located before a9 and after a9', respectively. The latter was detected only in one DF case. Although entailing two polar and non-charged amino acids, it was in a region described as relevant for NS5 interaction and NS4B dimerization, which turned it into a candidate for functional analysis testing.

Untranslated regions

Based on the already described secondary structures identified in the UTR regions flanking the ORF of the DENV genome (37-39), samples' sequences covering these genomic regions were used for secondary structure modelling. Particularly, samples 145 and 137 were employed as "baselines" for 5' and 3'UTR modelling, respectively, since they represent most of this study's sequences. These secondary structures prediction highly agreed with the topologies described by other authors (37, 39, 92-94).

Thirty variants previously detected within the UTRs (19) (Supplementary Table S1) were checked for secondary structure alterations. Stem-loops (SL) A and B on the 5'UTR, plus SLI and SLII accompanied by the short hairpins, dumbbell structures (DB) 1 and 2, the short hairpin (sHP) and the 3'SL structures on the 3'UTR were determined for the baseline consensus sequences and plotted on Fig 10. SNPs and iSNV's positions were mapped and denoted with grey arrows or grey stars, respectively. Their potential effect over the secondary structures was assessed, and it was shown that for 13 variants, a noticeable alteration was involved (Fig 10).

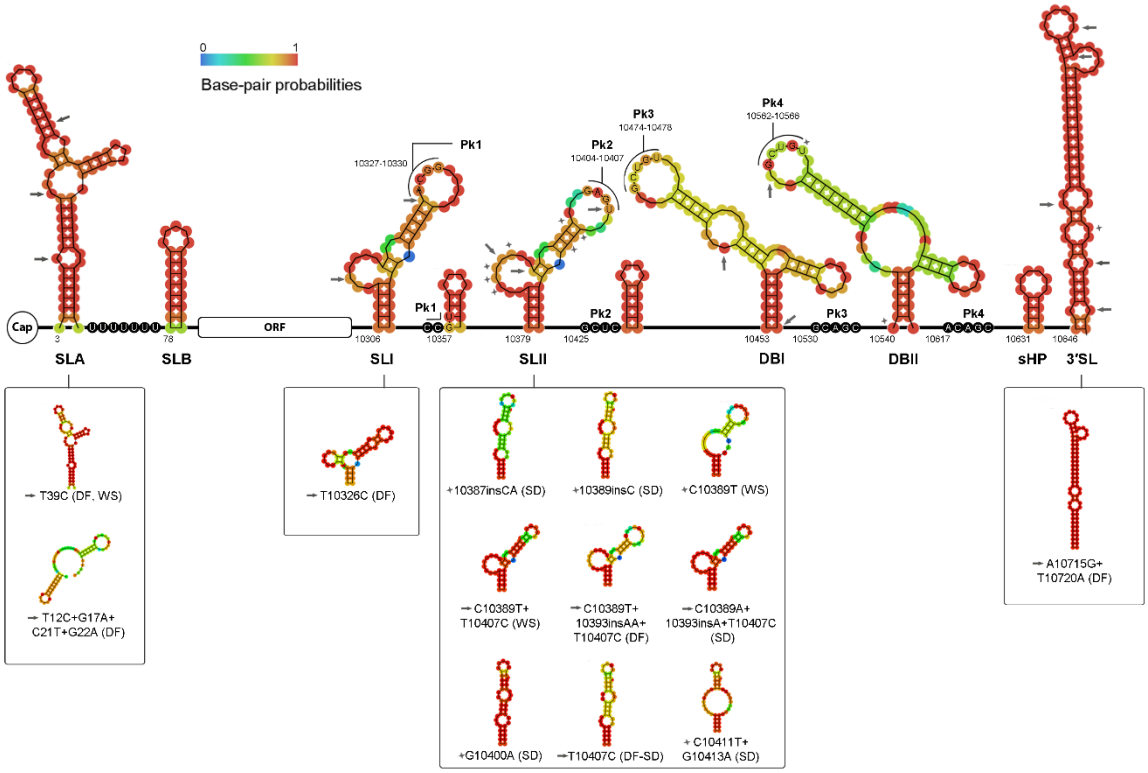


**FIG 10** Structural organization of DENV-2 UTRs. RNA secondary structures within the UTRs obtained with baseline sequences from samples 145 and 137 are denoted in the upper panel, where each circle represents a nucleotide and the color, its base pair probability. In regions where bases are not paired, the color represents the chance of staying unpaired. Bases involved in pseudoknots formations are denoted. Grey arrows and stars indicate the location of the different SNPs and iSNVs under study, respectively. The lower panel shows the structural disorders caused by the different variants within each respective secondary structure. SL: stem-loop; Pk: pseudoknot; DB: dumbbell-like; sHP: short hairpin; DF: dengue fever; WS: dengue with warning signs; SD: severe dengue.

On the 5'UTR and within the SLA, substitutions T10A and G17A detected at consensus level in a DF and a WS case, respectively, did not disturb the bulges present in this structure, and thus, consistently to Lodeiro and collaborators, viral replication would not be expected to be somehow affected (37). On the contrary, SNP T39C detected among DF+WS cases partially disrupted the top-stem stability and base-pairing, resembling the SLA structure displayed by the other DENV serotypes. It has been demonstrated before that DENV-2 polymerase could recognize and efficiently use the SLA of DENV1 both in vitro and in vivo (37), suggesting that this substitution might not cause any relevant impairment for viral replication. On the other side, the quadruple SNP substitution detected on one DF case shifted severely away from the typical structure, raising the number of possible different structures adopted in the thermodynamic ensemble, although with less stability (lower free energy) (Fig 10, Supplementary Table S5). Under these circumstances, it could be likely that a reduced efficacy in SLA's role for this sample could be responsible at some point for the milder clinical outcome of this case.

Regarding the variants located in the 3'UTR, only 11 seemed to cause a structural disorder. Curiously, 9 of them were located within the SLII (and mainly among WS+SD cases), while only one in the SLI. The latter (T10326C), detected at consensus level in one DF case, created a short hairpin in the bulge area, interrupting the top-stem area and rendering a different centroid/MFE structure with higher stability. On the other side, among the disruptive variants within the SLII, three were located in

the side loop spanning positions 10384-10392 (10387insCA, 10389insC and C10389T). However, although they disturbed this secondary structure, it has been shown before that the side loop of both SLs is under a relaxed selection, thus, tolerating high variability (39). It is important to notice that iSNV C10389T was also detected in other cases without causing any changes in SLII, which led to the suspicion that in this particular case, the structural alteration was, indeed, correlated with the one caused by consensus 10393delT carried by that particular sample. Substitutions C10389T or C10389A, however, when covaried with SNP T10407C, seemed to restore the disturbance caused by the latter, assuming the role of potential revertants. Mutation T10407C was incidentally detected at consensus level in almost half the samples (48.5%), a positive selection signal. Even though it altered the top-loop of SLII, it would strengthen the stability of the Pk2 structure by gaining an additional GC base pair. The remaining variants, iSNVs detected amid SD cases, located within the top-stem region of SLII, with G10400A prompting a similar secondary structure than T10407C, but with higher stability. Indeed, this structure represented 60% of the possible ones in the thermodynamical ensemble. The double iSNV C10411T+G10413A caused a base-pair disruption, creating a different secondary structure but of similar stability to the baseline (Fig 10, Supplementary Table S5). Furthermore, within the DBs structures, the only variant that requires attention was iSNV T10566C found in an SD case and located in the DB2 top-loop, specifically the region involved in Pk4 formation. The latter would decrease the Pk stability due to the loss of an interacting base pair. It has been proposed before that mutations in DB2 disrupting Pk interaction may provide a viral fitness advantage in the mosquito (95).

Although these results do not give a clear vision of the possible functional disorders caused by these mutations, it was curious that most of the variations located within these duplicated secondary structures were found mainly among cases of greater severity. Prior research suggested that higher variability is commonly detected in SLII due to mosquito-associated mutations, and that RNA duplication, i.e., SLI+SLII and DB1+DB2, is required for the virus to replicate in mammalian cells, despite the presence of SLII mutations that otherwise would be detrimental (39). If this scenario could be extrapolated to the in-vivo system, then the variants detected in SLII and DB2 would not be expected to impair viral viability within human hosts, despite causing structural alterations. It has been proposed that maintaining double copies of the RNA

structures is a viral strategy to ensure the functionality of one conserved element while the other is under different selective pressures in the two hosts (96). On the other hand, it has been demonstrated that SLII acts as a determinant for regulating subgenomic flavivirus RNA (sfRNA) production. These short non-coding RNAs are products of incomplete viral genome degradation by the host exonuclease Xrn1 in humans and mosquitoes and are relevant in viral pathogenesis and immune response evasion. Interestingly, viruses infecting mosquito cells generated a different pattern of sfRNAs than the infecting human cells, which was attributed to specific mutations in SLII, leading to the accumulation of shorter species of sfRNA (sfRNA3 and sfRNA4) during infection. When infecting human cells, these mosquito-adapted viruses displayed low fitness by inducing higher INF-I responses and were quickly outcompeted by viruses that generate the longer sfRNA1 (96). Even though the evidence might be indicating that SLII variants here detected could likely have arisen in the mosquito host and overcome transmission bottlenecks, their potential lower fitness in the human host would be counterintuitive with the fact that they were mainly found amid WS+SD cases. Functional assays would be fundamental to elucidate their natural effect on viral infection.

Finally, a double SNP variant (A10715G+T10720A) detected in one single DF case showed slightly disturbing the bottom half of the 3'SL, leading to two bulges' loss by creating a more stable stem with two additional base pairs. The bottom half of the 3'SL is a bulge-rich domain. It has been proposed that specific bulges might be required for optimal binding of cellular or viral proteins, which turns them into essential structures for viral replication (97). However, the lowest UU bulge spanning bases 10648 and 10720, when experimentally mutated to form a base pair in DENV-2, resulted in a slightly less efficient replication in monkey kidney cells but severely retarded in C6/36. On the contrary, bulges spanning bases 10656-10662 and 10707-10711, the two uppermost ones of the bottom half, showed to be essential for DENV-2 replication (92). It is not clear, though, whether the double SNP variant here detected could have affected viral replication within the patient. Nevertheless, if so, this could be a suitable explanation in line with this patient's favorable clinical outcome.

Detection of mutational hotspots

As mentioned in the previous section, assessing the presence of potential mutational hotspots in specific regions of the DENV-2 genome might be of major significance once they might be functional and related to any particular viral phenotype, or could simply represent a hallmark of immune evasion mechanisms, which ultimately could serve as valuable knowledge to intelligently improve the disease control. This way, by combining the mutation density analysis with the binomial test, five different mutational hotspots were identified: three amid DF cases, involving 18-bases regions within NS1, NS3 and 3'UTR, and two amid SD cases, within prM/M and 3'UTR (Fig 11) (a partial alignment of these hotspots can be found in Supplementary Figure S7). It is important to notice that these observations were consistent even after diminishing the windows size from 18 to 9 bases during the analysis, excepting for NS3 hotspot, which was exclusively detected with an 18-bases window. Also, it is crucial to highlight that both the mutational density and the binomial test were performed considering all substitutions and indels mapping within each genomic region. Thus, even though the density may seem high, or the p-value indicated that it was statistically significant for that particular region, not necessarily they would be representing mutational hotspots in the respective protein. However, it was remarkable the consistency between these results and the previously discussed findings regarding the relevant mutations detected in NS3 for DF and prM/M and 3'UTR for SD cases, with the latter coinciding exactly with the region of the SLII where the highest number of potentially disruptive mutations was found among WS+SD cases. Lastly, and looking forward, further research should certainly be carried on to explore the regions of higher mutational density, even though they were not statistically considered hotspots. Evidence suggested that the synonymous variations do not necessarily cause neutral changes. Instead, they could be related to selection processes that favor using certain codons that are mostly available in the human host cell (98-99). This selection of frequent codons is believed to occur mainly because common codons are translated more quickly (providing greater regulatory control) and with greater fidelity (producing more accurate protein sequences) than rare codons (100). Thus, highly expressed genes, as would be the case with the viral genome, will be enriched with common codons. Since the most significant influence of the use of codons is on the local translation rate, it could be thought that viral replication could adapt to the use of human codons,

optimizing their translation rate with a potential impact on protein expression and viral fitness.

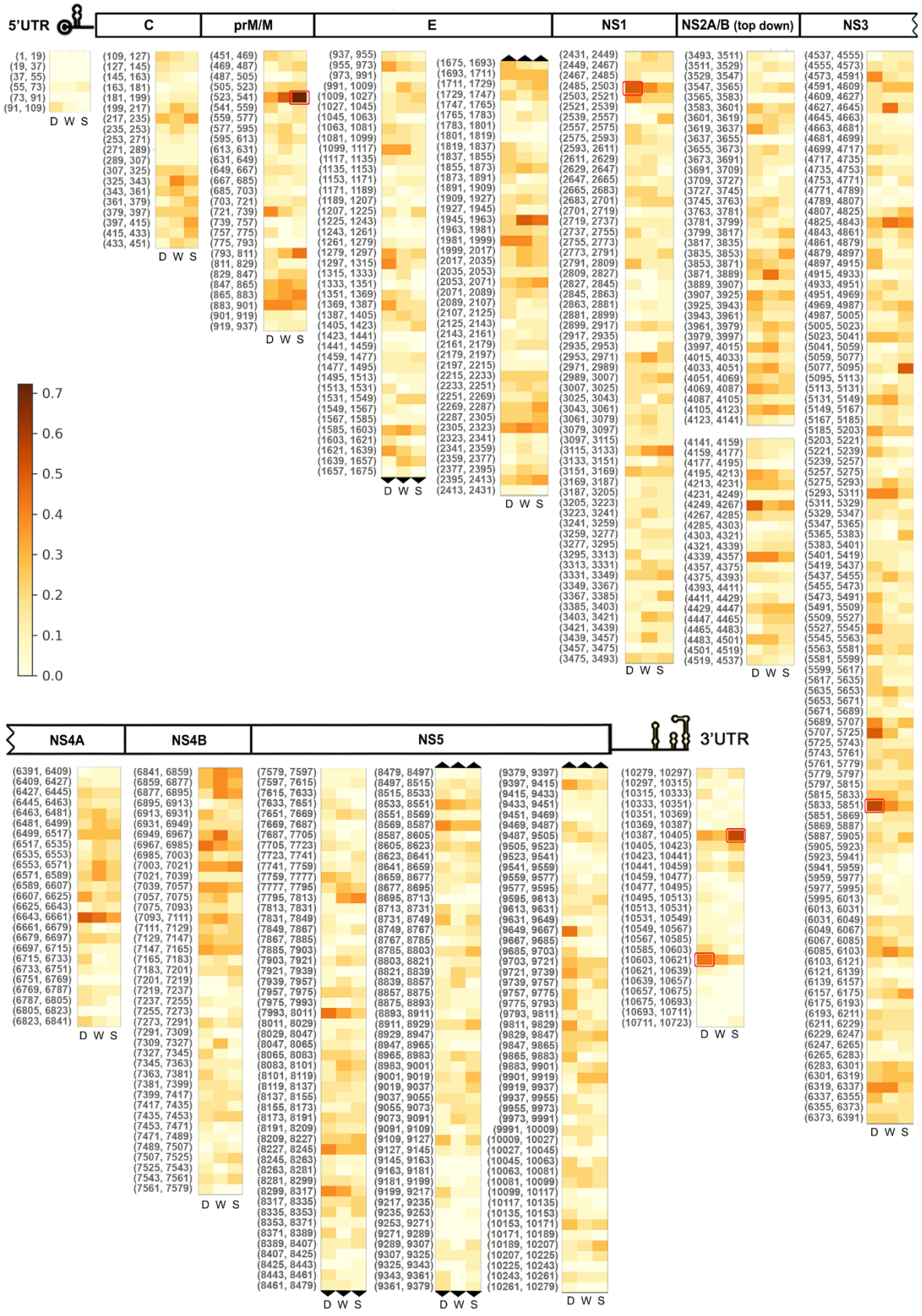


**FIG 11** Mutational burden assessment through mutational density and binomial testing. On this graph, each heat map represented the mutational density estimated within 18-bases non-overlapping sliding windows for each genomic region, considering indels, synonymous or non-synonymous substitutions, and ultimately computed for each clinical category, from left to right, D: DF cases, W: WS cases, and S: SD cases. Colored-scale indicates the density of accumulated mutations. Regions statistically considered as mutational hotspots are highlighted with a red rectangle.

Collectively, all the results exposed above showed that in general lines, disruptive variants were primarily identified among DF cases, while potentially immune-escape variants mainly were associated with WS+SD cases, which makes sense with the latter's longer intrahost evolution times. Upon confirming these variants' effect, they could be considered in the design of therapeutic or prophylactic compounds and the improvement of diagnostic assays. Although widely accepted, in-silico analysis suffers from some limitations, and under no circumstances, it replaces the functional ones through mutagenesis approaches. However, this study turned out to be essential to filter out the 141 mutations found in actual clinical cases, providing a crucial starting point to proceed towards functional assays with the relevant ones. Further research is undoubtedly required to disentangle the complex interactions between viral and host proteins. Numerous mutations located on the proteins' surface detected within this study require further analysis once there is a growing trend of studies reporting interactions with host proteins, critical for both immunomodulation and switching of the cellular machinery for viral use. Overall, this work provides new information about the implications of the intrahost genetic diversity of DENV-2, contributing to the knowledge about the viral factors possibly involved in its pathogenesis within the human host.

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## Competing interests

The authors declare no competing financial interests.

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Supplementary Information

**S1 Table -** Dataset of intrahost single nucleotide variants (iSNV) + single nucleotide polymorphisms detected at consensus level, selected for analysis. Nt: nucleotide; Aa: amino acid; DF: dengue fever cases; WS: cases with warning signs; SD: severe dengue cases; C: capsid; prM/M: membrane precursor; E: envelope; NS: non-structural protein.

| Gene | Nt substitution | Aa substitution | Status | DF cases [n (%)] | DF samples' ID | WS cases [n (%)] | WS samples' ID | SD cases [n (%)] | SD samples' ID | Primary cases | Primary samples' ID | Secondary cases | Secondary samples' ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | G125T | S10I | iSNV | - | | 1 (5.3) | 192 | - | | - | | 1(3.4) | 192 |
| | C219T | S10N | Consensus | 3(9.7) | 161, 168, 171 | - | | - | | 2(5.1) | 161, 171 | 1(3.4) | 168 |
| | C244T | L50F | Consensus | 2(6.5) | 146, 155 | - | | - | | - | | 2(6.9) | 146, 155 |
| | A307G | T71A | iSNV | 1(3.2) | 160 | - | | - | | - | | 1(3.4) | 160 |
| | G341T | R82I | iSNV | 15(48.4) | 162, 163, 166, 168, 169, 170, 171, 172, 173, 175, 177, 178, 179, 180, 184 | 2(10.5) | 185, 188 | 1(5.6) | 202 | 13(33.3) | 162,166,169,170,171,172,177,178,179,180,184,188,202 | 5(17.2) | 163,168,173,175,185 |
| | G406A | V104M | iSNV | - | | 1(5.3) | 193 | 1(5.6) | 200 | 1(2.6) | 193 | 1(3.4) | 200 |
| prM/M | A457C | N7H | iSNV | 12(38.7) | 163, 166, 168, 169, 171, 172, 174, 175, 177, 178, 182, 183 | 1(5.3) | 190 | - | | 9(23.1) | 166, 169, 171, 172, 174,177, 178, 182, 183 | 4(13.8) | 163,168,175,190 |
| | G481A | G15S | iSNV | - | | 1(5.3) | 205 | 2(11.1) | 195, 200 | 1(2.6) | 195 | 2(6.9) | 200,205 |
| | G523A | D29N | Consensus | - | | 2(10.5) | 188, 205 | 2(11.1) | 197, 204 | 3(7.7) | 188,197,204 | 1(3.4) | 205 |
| | | | iSNV | 2(6.5) | 168, 181 | 2(10.5) | 192, 156 | 4(22.2) | 208, 196, 200, 202 | 4(10.3) | 156,196,202,208 | 4(13.8) | 168,181,192,200 |
| | A555G | I39M | iSNV | - | | 1(5.3) | 205 | 2(11.1) | 197, 200 | 1(2.6) | 197 | 2(6.9) | 200,205 |
| | C880T | H148Y | iSNV | 1(3.2) | 181 | 2(10.5) | 192, 205 | 3(16.7) | 195, 197 ,200 | 2(5.1) | 159,197 | 4(13.8) | 181,192,200,205 |
| E | A1074G | I46M | iSNV | - | | 1(5.3) | 156 | - | | 1(2.6) | 156 | - | |
| | G1084A | A50T | iSNV | - | | 1(5.3) | 157 | - | | - | | 1(3.4) | 157 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C1094T | P53L | Consensus | 3(9.7) | 174, 177, 178 | - | | - | | 3(7.7) | 174, 177, 178 | - | |
| | | | iSNV | 4(12.9) | 163, 170, 173, 182 | 2(10.5) | 193, 205 | 9(50.0) | 209, 196, 198, 199, 200, 201, 202, 203, 204 | 8(20.5) | 170,182,193,196,198,202,204,209 | 7(24.1) | 163,173,199, 200,201,203, 205 |
| | A1148C | E71A | iSNV | - | | 1(5.3) | 205 | 2(11.1) | 195, 197 | 2(5.1) | 195,197 | 1(3.4) | 205 |
| | G1311A | M125I | iSNV | 14(45.2) | 163, 166, 169, 171, 172, 173, 174, 175, 177, 178, 179, 182, 183, 184 | 1(5.3) | 188 | - | | 11(28.2) | 169, 171, 172, 174, 177, 178, 179, 182, 183, 184,188 | 4(13.8) | 163,166,173, 175 |
| | G1396A | D154N | Consensus | - | | 1(5.3) | 188 | - | | 1(2.6) | 188 | - | |
| | | | iSNV | 1(3.2) | 181 | 1(5.3) | 205 | 4(22.2) | 196, 197, 200, 204 | 3(7.7) | 196, 197, 204 | 3(10.3) | 181,200,205 |
| | C1475T | T180I | Consensus | - | | 1(5.3) | 157 | - | | - | | 1(3.4) | 157 |
| | G1485A | M183I | Consensus | - | | 1(5.3) | 156 | - | | 1(2.6) | 156 | - | |
| | G1543A | D203N | iSNV | - | | 1(5.3) | 205 | 3(16.7) | 197, 198, 200 | 2(5.1) | 197,198 | 2(6.9) | 200,205 |
| | C1780T | H282Y | iSNV | 1(3.2) | 177 | - | | - | | 1(2.6) | 177 | - | |
| | T1834G | S300A | Consensus | 1(3.2) | 172 | - | | 1(5.6) | 201 | 1(2.6) | 172 | 1(3.4) | 201 |
| | | | iSNV | 2(6.5) | 163, 184 | - | | - | | 1(2.6) | 184 | 1(3.4) | 163 |
| | G1925A | G330D | iSNV | - | | 1(5.3) | 191 | - | | - | | 1(3.4) | 191 |
| | C1955T | T340I | iSNV | - | | 1(5.3) | 205 | 3(16.7) | 208, 195, 200 | 2(5.1) | 195,208 | 2(6.9) | 200,205 |
| | C1972T | H346Y | Consensus | - | | 2(10.5) | 188, 156 | 1(5.6) | 204 | 3(7.7) | 156,188,204 | - | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | iSNV | 2(6.5) | 168, 181 | 3(15.8) | 192, 205, 206 | 4(22.2) | 196, 197, 200, 202 | 3(7.7) | 196,197,202 | 6(20.7) | 168,181,192, 200,205,206 |
| | A2023G +G2024C | S363A | Consensus | 22(71.0) | 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184 | 2(10.5) | 190, 193 | - | | 17(43.6) | 161,162,166,167,169,170,171,172,174,177,178,179,180,182,183,184,193 | 7(24.1) | 160,163, 168, 173, 175,181, 190 |
| | | | iSNV | - | | 5(26.3) | 186, 189, 191, 192, 205 | 3(16.7) | 197, 203, 204 | 5(12.8) | 186,189,197,203,204 | 3(10.3) | 191,192,205 |
| | A2084G | E383G | iSNV | - | | - | | 1(5.6) | 143 | - | | 1(3.4) | 143 |
| | A2105G | N390S | Consensus | 2(6.5) | 162, 180 | - | | - | | 2(5.1) | 162, 180 | - | |
| | | | iSNV | - | | 1(5.3) | 191 | 1(5.6) | 198 | 1(2.6) | 198 | 1(3.4) | 191 |
| | C2393T | T486I | iSNV | - | | 1(5.3) | 190 | - | | - | | 1(3.4) | 190 |
| **NS1** | T2495A | V25E | iSNV | 4(12.9) | 141, 145, 151, 159 | 1(5.3) | 148 | - | | - | | 5(17.2) | 141, 145, 148, 151, 159 |
| | C2507T | T29I | Consensus | - | | - | | 1(5.6) | 143 | - | | 1(3.4) | 143 |
| | C2562A | Q47K | Consensus | 1(3.2) | 183 | - | | - | | 1(2.6) | 183 | - | |
| | C2791T | L124F | Consensus | 3(9.7) | 174, 177, 178 | - | | - | | 3(7.7) | 174, 177, 178 | - | |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | iSNV | 1(3.2) | 163 | 2(10.5) | 205, 206 | 5(27.8) | 196, 198, 199, 200, 201 | 2(5.1) | 196, 198 | 6(20.7) | 163,199, 200, 201,205,206 |
| | C2798T | T126I | Consensus | - | | - | | 1(5.6) | 140 | 1(2.6) | 140 | - | |
| | C2878A | L153M | Consensus | 22(71.0) | 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184 | 2(10.5) | 190, 193 | - | | 17(43.6) | 161, 162, 166, 167,169, 170, 171, 172,174, 177, 178, 179, 180, 182, 183, 184,193 | 7(24.1) | 160,163,168, 173,175,181, 190 |
| | | | iSNV | - | | 3(15.8) | 191, 207, 205 | 6(33.3) | 194, 195, 197, 198, 199, 200 | 4(10.3) | 194, 195, 197, 198 | 5(17.2) | 191,199,200, 207, 205 |
| | G2989A | D190N | Consensus | - | | 3(15.8) | 188, 205, 156 | 2(11.1) | 197, 204 | 4(10.3) | 156,188, 197, 204 | 1(3.4) | 205 |
| | | | iSNV | 2(6.5) | 168, 181 | 2(10.5) | 192, 193 | 5(27.8) | 195, 196, 199, 200, 202 | 4(10.3) | 193, 195, 196, 202 | 5(17.2) | 168,181,192, 199, 200 |
| | A3027G | I202M | Consensus | 1(3.2) | 179 | - | | - | | 1(2.6) | 179 | - | |
| | G3057A | M212I | iSNV | - | | 1(5.3) | 205 | 3(16.7) | 195, 197, 200 | 2(5.1) | 195,197 | 2(6.9) | 200,205 |
| | T3162A | F247L | Consensus | 3(9.7) | 173, 175, 179 | - | | - | | 1(2.6) | 179 | 2(6.9) | 173, 175 |
| | | | iSNV | - | | 1(5.3) | 191 | 3(16.7) | 208, 194, 201 | 2(5.1) | 194, 208 | 2(6.9) | 191,201 |
| | G3241GinsCCT | E274AT | iSNV | 17(54.8) | 160, 162, 166, 167, 168, 169, 170, 171, 173, 174, 175, 177, | 1(5.3) | 190 | 1(5.6) | 194 | 13(33.3) | 162, 166, 167, 169, 170, 171,174,177, 178, 179, 180, 184, 194 | 6(20.7) | 160,168,173, 175,181,190 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 178, 179, 180, 181, 184 | | | | | | | | |
| | G3289A | D290N | iSNV | - | | 1(5.3) | 192 | 2(11.1) | 197, 200 | 1(2.6) | 197 | 2(6.9) | 192,200 |
| | A3398G | E326G | iSNV | 13(41.9) | 160, 162, 167, 168, 169, 171, 172, 173, 174, 180, 181, 182, 183 | 1(5.3) | 185 | - | | 9(23.1) | 162, 167, 169, 171, 172, 174, 180, 182, 183 | 5(17.2) | 160,168,173, 181,185 |
| **NS2A** | A3562G | T29A | Consensus | 2(6.5) | 162, 180 | - | | - | | 2(5.1) | 162, 180 | - | |
| | | | iSNV | - | | - | | 2(11.1) | 194, 198 | 2(5.1) | 194, 198 | - | |
| | G3586A | A37T | Consensus | 1(3.2) | 184 | - | | - | | 1(2.6) | 184 | - | |
| | T3595C | F40L | Consensus | 3(9.7) | 174, 177, 178 | - | | - | | 3(7.7) | 174, 177, 178 | - | |
| | | | iSNV | 2(6.5) | 160, 182 | 2(10.5) | 193, 205 | 7(38.9) | 194, 196, 198, 199, 200, 201, 204 | 6(15.4) | 182,193, 194, 196, 198, 204 | 5(17.2) | 160,199,200, 201,205 |
| | A3664G | T63A | Consensus | 2(6.5) | 162, 180 | - | | - | | 2(5.1) | 162, 180 | - | |
| | | | iSNV | - | | 1(5.3) | 191 | - | | - | | 1(3.4) | 191 |
| | C3821T | T115I | Consensus | - | | 1(5.3) | 190 | - | | - | | 1(3.4) | 190 |
| | G3928C | A151P | iSNV | 16(51.6) | 162, 167, 168, 169, 170, 171, 172, 173, 174, 178, 179, 180, 181, 182, 183, 184 | 2(10.5) | 190, 193 | 1(5.6) | 200 | 14(35.9) | 162, 167,169, 170, 171, 172,174, 178, 179, 180, 182, 183, 184,193 | 5(17.2) | 168,173,181, 190,200 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C3935T | S153L | iSNV | 2(6.5) | 168, 181 | 2(10.5) | 192, 205 | 4(22.2) | 195, 197, 200, 202 | 3(7.7) | 195, 197, 202 | 5(17.2) | 168, 181,192,200, 205 |
| | A4034G | Q186R | Consensus | 13(41.9) | 160, 163, 167, 169, 171, 172, 174, 177, 178, 181, 182, 183, 184 | 2(10.5) | 190, 193 | - | | 11(28.2) | 167, 169, 171, 172, 174, 177, 178, 182, 183, 184,193 | 4(13.8) | 160,163,181, 190 |
| | | | iSNV | 2(6.5) | 162, 166 | 5(26.3) | 189, 192, 207, 205, 206 | 9(50.0) | 208, 194, 195, 196, 197, 199, 202, 203, 204 | 11(28.2) | 162,166,189, 194, 195, 196, 197, 202, 203, 204, 208 | 5(17.2) | 192,199, 205, 206,207 |
| | G4042A | A189T | Consensus | - | | 1(5.3) | 190 | - | | - | | 1(3.4) | 190 |
| | G4042T | A189S | iSNV | - | | - | | 1(5.6) | 198 | 1(2.6) | 198 | - | |
| | G4121A | S215N | iSNV | 1(3.2) | 181 | 4(21.1) | 192, 193, 207, 205 | 4(22.2) | 209, 195, 197, 200 | 5(12.8) | 193, 195, 197, 200, 209 | 4(13.8) | 181,192,205, 207 |
| **NS3** | G4562A | G14E | Consensus | 3(9.7) | 172, 158, 159 | - | | - | | 3(7.7) | 172, 158, 159 | - | |
| | G5026A | E169K | iSNV | 5(16.1) | 166, 168, 170, 177, 178 | 1(5.3) | 186 | 1(5.6) | 209 | 6(15.4) | 166,170,177,178,186,209 | 1(3.4) | 168 |
| | C5304G/T5304G | C261W | iSNV | 15(48.4) | 166, 168, 170, 173, 180, 154, 155, 158, 169, 171, 172, 174, 177, 178, 184 | 3(15.9) | 185, 188, 193 | 1(5.6) | 202 | 13(33.3) | 166, 169,170, 171, 172, 174, 177, 178, 180, 184, 188, 193, 202 | 6(20.7) | 154, 155, 158, 168,173,185 |
| | C5309A | A263D | iSNV | 4(12.9) | 168, 170, 173, 180 | 3(15.8) | 185, 186, 188 | - | | 4(10.3) | 170,180,186,188 | 3(10.3) | 168,173,185 |
| | G5531T | R337I | iSNV | 14(45.2) | 166, 167, 168, 169, 170, 171, | 4(21.1) | 185, 186, 188, 144 | 3(16.7) | 202, 203, 139 | 16(41.0) | 139,144, 166, 167,169, 170, | 6(20.7) | 168,175,181, 185,202,203 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 172, 174, 175, 178, 179, 180, 181, 183, 184 | | | | | | 171, 172, 174,178, 179, 180, 183, 184,186,188 | | |
| | G5642T | C374F | iSNV | 4(12.9) | 166, 172, 177, 180 | 2(10.5) | 185, 188 | - | | 5(12.8) | 166, 172, 177, 180,188 | 1(3.4) | 185 |
| | G6000A | M493I | Consensus | 1(3.2) | 180 | - | | - | | 1(2.6) | 180 | - | |
| **NS4A** | T6496C | Y41H | Consensus | 22(71.0) | 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184 | 2(10.5) | 190, 193 | - | | 17(43.6) | 161, 162, 166, 167, 169, 170, 171, 172,174, 177, 178, 179, 180, 182, 183, 184,193 | 7(24.1) | 160,163,168, 173,175,181, 190 |
| | | | iSNV | - | | 3(15.8) | 191, 207, 205 | 9(50.0) | 194, 195, 196, 197, 198, 199, 200, 203, 204 | 7(17.9) | 194, 195, 196, 197, 198,203, 204 | 5(17.2) | 191,199,200, 205,207 |
| | C6500A | T42N | iSNV | - | | 3(15.8) | 191, 207, 205 | 9(50.0) | 194, 195, 196, 197, 198, 199, 200, 203, 204 | 7(17.9) | 194, 195, 196, 197, 198, 203, 204 | 5(17.2) | 191,199,200, 205,207 |
| | | | Consensus | 22(71.0) | 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184 | 2(10.5) | 190, 193 | - | | 17(43.6) | 161, 162, 166, 167, 169, 170, 171, 172,174, 177, 178, 179, 180, 182, 183, 184,193 | 7(24.1) | 160,163,168, 173,175,181, 190 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G6512A | S46N | Consensus | - | | 2(10.5) | 188, 205 | 6(33.3) | 196, 197, 200, 204, 137, 143 | 6(15.4) | 137, 143,188, 196, 197, 204, | 2(6.9) | 200,205 |
| | | | iSNV | 2(6.5) | 168, 181 | 2(10.5) | 192, 206 | 4(22.2) | 194, 198, 199, 202 | 3(7.7) | 194, 198,202 | 5(17.2) | 168, 181,192,199, 206 |
| | G6562A | A63T | Consensus | 22(71.0) | 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184 | 3(15.8) | 190, 191, 193 | - | | 17(43.6) | 161, 162, 166, 167, 169, 170, 171, 172,174, 177, 178, 179, 180, 182, 183, 184,193 | 8(27.6) | 160,163,168, 173,175,181, 190,191 |
| | | | iSNV | - | | 2(10.5) | 207, 205 | 9(50.0) | 194, 195, 196, 197, 198, 199, 200, 203, 204 | 7(17.9) | 194, 195, 196, 197, 198,203, 204 | 4(13.8) | 199, 200, 205,207 |
| **NS4B** | G6844A | E7K | iSNV | 16(51.6) | 161, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 154 | 6(31.6) | 185,186, 187, 188, 189, 190 | 2(11.1) | 202, 149 | 17(43.6) | 149, 161, 166, 167, 169, 170, 171, 172, 174, 177, 178, 179, 180,186,188,189,202 | 7(24.1) | 154,168,173, 175,185,187, 190 |
| | C6862T | L13F | iSNV | 1(3.2) | 181 | 1(5.3) | 205 | 3(16.7) | 195, 197, 200 | 2(5.1) | 195, 197 | 3(10.3) | 181,200,205 |
| | T6870G | F15L | iSNV | 1(3.2) | 181 | 1(5.3) | 205 | 4(22.2) | 209, 195, 197, 200 | 3(7.7) | 195, 197,209 | 3(10.3) | 181,200,205 |
| | G6871A | G16R | Consensus | 4(12.9) | 145 151 154 159 | 9(4.7) | 147 148 153 185 186 187 189 192 207 | 1(5.6) | 149 | 4(10.3) | 149,153,186,189 | 10(34.5) | 145,147,148, 151, 154, 159,185,187, 192,207 |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | iSNV | 5(16.1) | 161, 167, 181, 182, 183 | 3(15.8) | 191, 206, 156 | 10(55.6) | 208, 209, 194, 195, 196, 198, 199, 201, 203, 204 | 13(33.3) | 156, 161, 167, 182, 183, 194, 195, 196, 198, 203, 204,208, 209 | 5(17.2) | 181,191,199, 201,206 |
| | A6880G | T19A | iSNV | - | | 1(5.3) | 205 | 4(22.2) | 209, 195, 197, 200 | 3(7.7) | 195, 197, 209 | 2(6.9) | 200,205 |
| | C6884T | T20I | Consensus | 2(6.5) | 159 154 | 3(15.8) | 192 187 153 | - | | 1(2.6) | 153 | 4(13.8) | 154,159,187, 192 |
| | | | iSNV | - | | - | | 2(11.1) | 209, 199 | 1(2.6) | 209 | 1(3.4) | 199 |
| | G6889C | E22Q | iSNV | - | | 1(5.3) | 205 | 3(16.7) | 195, 197, 200 | 2(5.1) | 195,197 | 2(6.9) | 200,205 |
| | T6892C | S23P | iSNV | - | | 1(5.3) | 205 | 3(16.7) | 195, 197, 200 | 2(5.1) | 195,197 | 2(6.9) | 200,205 |
| | C6922T | R33C | iSNV | 14(45.2 | 160, 166, 168, 169, 170, 171, 173, 174, 175, 177, 178, 179, 180, 181 | 7(36.8) | 185,186, 187, 188, 189, 190, 193 | 1(5.6) | 202 | 14(35.9) | 166, 169, 170, 171, 174, 177, 178, 179, 180, 186,188,189,193,202 | 8(27.6) | 160,168,173, 175,181,185, 187,190 |
| | A6958G | T45A | iSNV | 9(29.0) | 162, 166, 168, 169, 170, 171, 173, 175, 180 | 2(10.5) | 188, 190 | - | | 7(17.9) | 162, 166, 169, 170, 171, 180,188 | 4(13.8) | 168,173,175, 190 |
| | T7049C | L75S | iSNV | 8(25.8) | 169, 171, 172, 174, 175, 177, 178, 179 | 5(26.3) | 185,186, 187, 188, 189, | 1(5.6) | 139 | 12(30.8) | 139, 169, 171, 172, 174, 175, 177, 178, 179,186,188,189 | 3(10.3) | 175,185,187 |
| | A7471G | T216A | iSNV | 4(12.9) | 168, 169, 171, 172 | 1(5.3) | 189 | - | | 4(10.3) | 169, 171, 172, 189 | 1(3.4) | 168 |
| | A7559G | N245S | Consensus | 1(3.2) | 158 | - | | - | | - | | 1(3.4) | 158 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **NS5** | C7614A | S15R | Consensus | 1(3.2) | 175 | - | | - | | - | | 1(3.4) | 175 |
| | C7627A | L20M | Consensus | - | | 1(5.3) | 148 | - | | - | | 1(3.4) | 148 |
| | C7645A | Q26K | iSNV | 12(38.7) | 163, 166, 168, 169, 170, 171, 172, 173, 175, 177, 178, 179 | 1(5.3) | 188 | - | | 9(23.1) | 166, 169, 170, 171, 172, 177, 178, 179,188 | 4(13.8) | 163,168,173, 175 |
| | T7649C | I27T | Consensus | - | | 4(21.1) | 186, 189, 147, 148 | 1(5.6) | 149 | 3(7.7) | 149, 186, 189 | 2(6.9) | 147, 148 |
| | T7763C | F65S | iSNV | 5(16.1) | 162, 166, 173, 175, 180 | - | | - | | 3(7.7) | 162, 166, 180 | 2(6.9) | 173, 175 |
| | A7777C | M70L | iSNV | - | | 2(10.5) | 192, 205 | 2(11.1) | 195, 197 | 2(5.1) | 195, 197 | 2(6.9) | 192, 205 |
| | C7976A | P136Q | Consensus | 1(3.2) | 158 | - | | - | | - | | 1(3.4) | 158 |
| | | | iSNV | - | | - | | 1(5.6) | 194 | 1(2.6) | 194 | - | |
| | G8074C | E169Q | iSNV | 17(54.8) | 161, 163, 166, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 183 | 2(10.5) | 188, 190 | 1(5.6) | 202 | 14(35.9) | 161, 166,169, 170, 171, 172, 174, 177, 178, 179, 180, 183,188,202 | 6(20.7) | 163,168,173, 175,181,190 |
| | G8089A | D174N | iSNV | - | | 1(5.3) | 192 | - | | - | | 1(3.4) | 192 |
| | A8155G | T196A | iSNV | - | | - | | 1(5.6) | 197 | 1(2.6) | 197 | - | |
| | T8369C | I267T | Consensus | - | | - | | 1(5.6) | 202 | 1(2.6) | 202 | - | |
| | T8381C | I271T | Consensus | - | | 1(5.3) | 205 | - | | - | | 1(3.4) | 205 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A8698G | T377A | Consensus | - | | 1(5.3) | 157 | - | | - | | 1(3.4) | 157 |
| | G8709T | W380C | Consensus | - | | - | | 1(5.6) | 197 | 1(2.6) | 197 | - | |
| | A8746G | M393V | iSNV | - | | - | | 1(5.6) | 194 | 1(2.6) | 194 | - | |
| | G8859T | R430S | Consensus | - | | 3(15.8) | 188, 205, 156 | 2(11.1) | 137, 143 | 3(7.7) | 137,156,188 | 2(6.9) | 143,205 |
| | | | iSNV | 1(3.2) | 181 | - | | 5(27.8) | 208, 196, 197, 200, 204 | 5(12.8) | 196, 197, 200, 204, 208 | 1(3.4) | 181 |
| | G9071A | G501E | iSNV | - | | 1(5.3) | 205 | 2(11.1) | 195, 200 | 1(2.6) | 195 | 2(6.9) | 200,205 |
| | C9322T | P585S | Consensus | 6(19.4) | 160, 163, 167, 181, 182, 183 | 1(5.3) | 193 | - | | 4(10.3) | 167,182,183,193 | 3(10.3) | 160, 163,181 |
| | | | iSNV | 1(3.2) | 166 | - | | - | | 1(2.6) | 166 | - | |
| | A9416G | E616G | iSNV | - | | 1(5.3) | 205 | - | | - | | 1(3.4) | 205 |
| | T9491C | I641T | Consensus | - | | 4(21.1) | 186, 189, 147, 148 | 1(5.6) | 149 | 3(7.7) | 149, 186, 189 | 2(6.9) | 147, 148 |
| | | | iSNV | - | | 1(5.3) | 206 | 1(5.6) | 198 | 1(2.6) | 198 | 1(3.4) | 206 |
| | G9501C | Q644H | iSNV | - | | 4(21.1) | 191, 192, 207, 205 | 5(27.8) | 196, 197, 198, 203, 204 | 5(12.8) | 196, 197, 198, 203, 204 | 4(13.8) | 191, 192, 207, 205 |
| | | | Consensus | 22(71.0) | 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184 | 2(10.5) | 190, 193 | - | | 17(43.6) | 161, 162, 166, 167, 169, 170, 171, 172,174, 177, 178, 179, 180, 182, 183, 184,193 | 7(24.1) | 160,163,168, 173,175,181, 190 |
| | A9595G | S676G | iSNV | - | | - | | 1(5.6) | 197 | 1(2.6) | 197 | - | |
| | C9605T | T679I | iSNV | - | | 1(5.3) | 191 | - | | - | | 1(3.4) | 191 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A9725T | K719I | iSNV | 1(3.2) | 184 | 2(10.5) | 191, 206 | 2(11.1) | 208, 194 | 3(7.7) | 184,194,208 | 2(6.9) | 191, 206 |
| | | | Consensus | 1(3.2) | 166 | - | | - | | 1(2.6) | 166 | - | |
| | A9814G | K749E | iSNV | 1(3.2) | 171 | - | | - | | 1(2.6) | 171 | - | |
| | G10060A | E831K | Consensus | - | | - | | 1(5.6) | 202 | 1(2.6) | 202 | - | |
| | G10202A | G878E | Consensus | 22(71.0) | 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184 | 2(10.5) | 190, 193 | 1(5.6) | 204, | 18(46.2) | 161, 162, 166, 167, 169, 170, 171, 172,174, 177, 178, 179, 180, 182, 183, 184,193,204 | 7(24.1) | 160,163,168, 173,175,181, 190 |
| | | | iSNV | - | | 4(21.1) | 186, 189, 192, 205 | 3(16.7) | 197, 202, 203 | 5(12.8) | 186,189, 197, 202, 203 | 2(6.9) | 192,205 |
| | G10252A | E895K | Consensus | 21(67.7) | 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184 | 1(5.3) | 190 | 2(11.1) | 197, 204 | 18(46.2) | 161, 162, 166, 167, 169, 170, 171, 172, 174,177, 178, 179, 180, 182, 183, 184, 197, 204 | 7(24.1) | 160,163,168, 173,175,181, 190 |
| | | | iSNV | - | | 4(21.1) | 186, 191, 192, 205 | 2(11.1) | 200, 203 | 2(5.1) | 186,203 | 4(13.8) | 191,192,200, 205 |
| **5'UTR** | T10A | | Consensus | 1(3.2) | 163 | - | | - | | - | | 1(3.4) | 163 |
| | T12C | | Consensus | 1(3.2) | 167 | - | | - | | 1(2.6) | 167 | - | |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G17A | | Consensus | 1(3.2) | 167 | 1(5.3) | 157 | - | | 1(2.6) | 167 | 1(3.4) | 157 |
| | C21T | | Consensus | 1(3.2) | 167 | - | | - | | 1(2.6) | 167 | - | |
| | G22A | | Consensus | 1(3.2) | 167 | - | | - | | 1(2.6) | 167 | - | |
| | T39C | | Consensus | 2(6.5) | 154, 159 | 3(15.8) | 153, 187, 192 | - | | 1(2.6) | 153 | 4(13.8) | 154, 159,187,192 |
| **3'UTR** | G10313A | | Consensus | 1(3.2) | 182 | - | | - | | 1(2.6) | 182 | - | |
| | T10326C | | Consensus | 1(3.2) | 162 | - | | - | | 1(2.6) | 162 | - | |
| | T10386TinsCA | | iSNV | - | | - | | 1(5.6) | 208 | 1(2.6) | 208 | - | |
| | C10387CinsAT | | iSNV | - | | - | | 1(5.6) | 208 | 1(2.6) | 208 | - | |
| | C10387T | | iSNV | - | | - | | 1(5.6) | 195 | 1(2.6) | 195 | - | |
| | A10388AinsC | | iSNV | - | | - | | 1(5.6) | 208 | 1(2.6) | 208 | - | |
| | C10389T | | Consensus | 1(3.2) | 166 | 1(5.3) | 206 | - | | 1(2.6) | 166 | 1(3.4) | 206 |
| | | | iSNV | 1(3.2) | 184 | 1(5.3) | 191 | 2(11.1) | 194,195 | 3(7.7) | 184,194,195 | 1(3.4) | 191 |
| | C10389A | | Consensus | 1(3.2) | | 1(5.3) | 208 | - | | 1(2.6) | 208 | - | |
| | C10389CinsA | | iSNV | 5(16.1) | 160,163,167,181,184 | 4(21.1) | 191,192, 193,207 | 9(50.0) | 194,195,196,197,198,200,201,203,204 | 10(25.6) | 167,184,193,194,195,196,197,198,203,204 | 8(27.6) | 160,163,181, 191,192,200, 201,207 |
| | C10389CinsAA | | iSNV | - | | 2(10.5) | 191,205 | 7(38.9) | 194,195,196,197,198,200,204 | 6(15.4) | 194,195,196,197,198,204 | 3(10.3) | 191,200,205 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10390delA | | iSNV | 9(29.0) | 160,161,162,163,166,167,168,169,170,171,172,173,174,175,177,178,179,180,181,183,184 | 2(10.5) | 190, 193 | 2(11.1) | 209,201 | 8(20.5) | 161,162,167,169,182,184,193,209 | 5(17.2) | 160,163,181,190,201 |
| | 10390delAA | | iSNV | 5(16.1) | 161,162,168,170,173 | - | | - | | 3(7.7) | 161,162,170 | 2(6.9) | 168,173 |
| | 10393delT | | Consensus | - | | 1(5.3) | 191 | - | | - | | 1(3.4) | 191 |
| | 10393insA | | Consensus | 9(29.0) | 161, 162, 166, 168, 170, 173, 175, 179, 180 | - | | - | | 6(15.4) | 161, 162, 166, 170, 179, 180 | 3(10.3) | 168,173,175 |
| | 10393insAA | | Consensus | 22(71.0) | 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184 | 1(5.3) | 193 | 2(11.1) | 201,209 | 18(46.2) | 161, 162, 166, 167, 169, 170, 171, 172,174, 177, 178, 179, 180, 182, 183, 184,193,209 | 7(24.1) | 160,163,168,173,175,181,201 |
| | G10400A | | iSNV | - | | - | | 2(11.1) | 195,200 | 1(2.6) | 195 | 1(3.4) | 200 |
| | T10407C | | Consensus | 22(71.0) | 160,161,162,163,166,167,168,169,170,171,172,173,174,175,177,178,179,180,181,183,184 | 4(21.1) | 157,190,193,206 | 7(38.9) | 140, 194,195,198,201,208,209 | 22(56.4) | 140,161,162,166,167,169,170,171,172,174,177,178,179,180,183,184,193,194,195,198,208,209 | 10(34.5) | 157,160,163,168,173,175,181,190,201,206 |
| | C10411T | | iSNV | - | | - | | 2(11.1) | 195, 200 | 1(2.6) | 195 | 1(3.4) | 200 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G10413A | | iSNV | - | | - | | 2(11.1) | 195, 200 | 1(2.6) | 195 | 1(3.4) | 200 |
| | A10463G | | iSNV, Consensus | - | | - | | 2(11.1) | 195, 202 | 2(5.1) | 195, 202 | - | |
| | C10520A | | Consensus | 1(3.2) | 184 | - | | - | | 1(2.6) | 184 | - | |
| | C10540T | | iSNV | - | | - | | 1(5.6) | 195 | 1(2.6) | 195 | - | |
| | A10563G | | Consensus | 1(3.2) | 170 | - | | - | | 1(2.6) | 170 | - | |
| | T10566C | | iSNV | - | | - | | 1(5.6) | 137 | 1(2.6) | 137 | - | |
| | C10660T | | Consensus | - | | - | | 1(5.6) | 140 | 1(2.6) | 140 | - | |
| | C10684A | | Consensus | - | | 1(5.3) | 156 | - | | 1(2.6) | 156 | - | |
| | C10687T | | Consensus | - | | - | | 1(5.6) | 149 | 1(2.6) | 149 | - | |
| | A10711T | | iSNV | - | | - | | 1(5.6) | 149 | 1(2.6) | 149 | - | |
| | A10715G | | Consensus | 1(3.2) | 162 | - | | - | | 1(2.6) | 162 | - | |
| | T10720A | | Consensus | 1(3.2) | 162 | - | | - | | 1(2.6) | 162 | - | |

**S2 Table** - Consensus sequences clustering after alignment with ClustalW and CD-HIT. One sequence of each cluster was then randomly selected for molecular modelling. C: capsid; prM/M: membrane precursor; E: envelope; NS: non-structural protein.

| Gene | Cluster | Samples' ID |
|---|---|---|
| C | Cluster 1 | 137, 138, 139, 143, 144, 145,147, 148, 149,151, 153, 154, 157, 159, 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 179, 180, 181, 182, 183, 184, 185, 186, 187, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 203, 206, 207, 208, 209 |
| | Cluster 2 | 141, 142 |
| | Cluster 3 | 146, 155 |
| | Cluster 4 | 156, 188, 204, 205 |
| | Cluster 5 | 158 |
| | Cluster 6 | 174, 177, 178 |
| | | |
| prM/M | Cluster 1 | 137, 138, 139, 140, 141, 142, 143, 144, 146, 147, 148, 149, 151, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 189, 191, 192, 193, 194, 195, 196, 198, 199, 200, 201, 202, 203, 206, 208, 209 |
| | Cluster 2 | 145 |
| | Cluster 3 | 188, 197, 204, 205 |
| | Cluster 4 | 190 |
| | Cluster 5 | 207 |
| | | |
| E | Cluster 1 | 137,139, 140, 141, 142, 144, 145, 146, 147, 147, 148, 149, 151, 153, 154, 155, 158, 159, 185, 186, 187,189, 194, 195, 196, 197, 198, 199, 200, 202, 205, 206, 207, 208, 209 |
| | Cluster 2 | 138 |
| | Cluster 3 | 143 |
| | Cluster 4 | 156 |
| | Cluster 5 | 157 |
| | Cluster 6 | 160, 163, 167, 171, 181, 12, 183, 184, 193 |
| | Cluster 7 | 161, 166, 168, 170, 173, 175, 179, |
| | Cluster 8 | 162, 180 |
| | Cluster 9 | 172 |
| | Cluster 10 | 174, 177, 178 |
| | Cluster 11 | 188 |
| | Cluster 12 | 190 |
| | Cluster 13 | 191 |
| | Cluster 14 | 202, 203, 204, 205, 206, 207, 208, 209 |
| | | |
| NS1 | Cluster 1 | 137, 139, 141, 142, 144, 146, 151, 155, 157, 185, 191, 194, 195, 196, 198, 199, 200, 202, 206, 207, 208, 209 |

| | | |
|---|---|---|
| | Cluster 2 | 138 |
| | Cluster 3 | 140 |
| | Cluster 4 | 143 |
| | Cluster 5 | 145, 147, 148, 149, 153, 154, 155, 186, 187, 189, 192 |
| | Cluster 6 | 156, 188, 197, 204, 205, |
| | Cluster 7 | 158 |
| | Cluster 8 | 160, 161, 162, 163, 166, 167, 168, 170, 171, 180, 181, 182, 184, 190, 193 |
| | Cluster 9 | 169 |
| | Cluster 10 | 172 |
| | Cluster 11 | 173, 175 |
| | Cluster 12 | 174, 177, 178 |
| | Cluster 13 | 179 |
| | Cluster 14 | 183 |
| | Cluster 15 | 201 |
| | Cluster 16 | 203 |
| | | |
| NS2A | Cluster 1 | 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 151, 153, 154, 155, 156, 157, 159, 185, 186, 187, 188, 189, 191, 192, 194, 195, 196, 197, 198, 199, 200, 201, 203, 204, 205, 206, 207, 208, 209 |
| | Cluster 2 | 158 |
| | Cluster 3 | 160, 163, 167, 181, 182, 183, 193 |
| | Cluster 4 | 161, 166, 168, 170, 173, 175, 179 |
| | Cluster 5 | 162, 180 |
| | Cluster 6 | 169 |
| | Cluster 7 | 171, 172 |
| | Cluster 8 | 174, 177, 178 |
| | Cluster 9 | 184 |
| | Cluster 10 | 190 |
| | | |
| NS2B | Cluster 1 | 137, 138, 139, 140, 141, 142, 143, 144, 146, 147, 148, 149, 151, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 174, 177, 178, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209 |
| | Cluster 2 | 173, 175, 179 |
| | Cluster 3 | 196 |
| | | |
| NS3 | Cluster 1 | 137, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 151, 153, 154, 155, 156, 157, 185, 186, 187, 188, 189, 191, 192, 195, 197, 198, 199, 200, 202, 203, 204, 206, 207, 208, 209 |
| | Cluster 2 | 138 |
| | Cluster 3 | 158, 159 |
| | Cluster 4 | 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 173, 174, 175, 177, 178, 179, 181, 182, 183, 184, 193 |
| | Cluster 5 | 172 |
| | Cluster 6 | 180 |

| | | |
|---|---|---|
| | Cluster 7 | 190 |
| | Cluster 8 | 194 |
| | Cluster 9 | 196 |
| | Cluster 10 | 201 |
| | Cluster 11 | 205 |
| | | |
| NS4A | Cluster 1 | 137, 143, 188, 196, 197, 200, 204, 205, |
| | Cluster 2 | 138, 139, 140, 141, 142, 144, 145, 146, 147, 148, 149, 151, 153, 154, 155, 156, 157, 158, 159, 185, 186, 187, 189, 192, 195, 198, 199, 201, 202, 203, 206, 207, 208, 209 |
| | Cluster 3 | 160, 161, 162, 163, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184, 190, 193 |
| | Cluster 4 | 166 |
| | Cluster 5 | 191 |
| | Cluster 6 | 194 |
| | | |
| NS4B | Cluster 1 | 137, 138, 139, 140, 141, 142, 143, 144, 146, 155, 157, 160, 161, 162, 163, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 177, 178, 179, 180, 181, 182, 183, 184, 190, 191, 193, 195, 196, 198, 199, 200, 201, 202, 203, 206, 208, 209 |
| | Cluster 2 | 145, 147, 148, 149, 151, 185, 186, 189, 207 |
| | Cluster 3 | 153, 154, 159, 187, 192 |
| | Cluster 4 | 156, 188, 197, 204, 205 |
| | Cluster 5 | 158 |
| | Cluster 6 | 194 |
| | | |
| NS5 | Cluster 1 | 138, 139, 141, 142, 144, 155, 191 |
| | Cluster 2 | 153, 154, 159, 187, 192 |
| | Cluster 3 | 147, 149, 186, 189 |
| | Cluster 4 | 171, 172, 174, 178 |
| | Cluster 5 | 137, 143 |
| | Cluster 6 | 145, 185 |
| | Cluster 7 | 168, 170 |
| | Cluster 8 | 173, 179 |
| | Cluster 9 | 140 |
| | Cluster 10 | 146 |
| | Cluster 11 | 148 |
| | Cluster 12 | 151 |
| | Cluster 13 | 156 |
| | Cluster 14 | 157 |
| | Cluster 15 | 158 |
| | Cluster 16 | 160 |
| | Cluster 17 | 161 |
| | Cluster 18 | 162 |
| | Cluster 19 | 163 |

| | | |
|---|---|---|
| | Cluster 20 | 166 |
| | Cluster 21 | 167 |
| | Cluster 22 | 169 |
| | Cluster 23 | 175 |
| | Cluster 24 | 177 |
| | Cluster 25 | 180 |
| | Cluster 26 | 181 |
| | Cluster 27 | 182 |
| | Cluster 28 | 183 |
| | Cluster 29 | 184 |
| | Cluster 30 | 188 |
| | Cluster 31 | 190 |
| | Cluster 32 | 193 |
| | Cluster 33 | 194 |
| | Cluster 34 | 195 |
| | Cluster 35 | 196 |
| | Cluster 36 | 197 |
| | Cluster 37 | 198 |
| | Cluster 38 | 199 |
| | Cluster 39 | 200 |
| | Cluster 40 | 201 |
| | Cluster 41 | 202 |
| | Cluster 42 | 203 |
| | Cluster 43 | 204 |
| | Cluster 44 | 205 |
| | Cluster 45 | 206 |
| | Cluster 46 | 207 |
| | Cluster 47 | 208 |
| | Cluster 48 | 209 |

**S3 Table** - Templates used for comparative modelling.

| Target | PDB ID | Method | Resolution (Å) | R-value free | Outliers |
|---|---|---|---|---|---|
| C | 6VG5 | X-Ray | 1.5 | 0.194 | 0.00% |
| prM/M | 3C5X | X-Ray | 2.2 | 0.275 | 0.70% |
| | 7BUD | Electron Microscopy | 4.5 | - | 5.30% |
| E | 4UT6 | X-Ray | 3.2 | 0.278 | 1.60% |
| NS1 | 4O6B | X-Ray | 3 | 0.219 | 0.00% |
| NS3 | 5YW1 | X-Ray | 2.6 | 0.24 | 0.60% |
| NS5 | 5ZQK | X-Ray | 2.3 | 0.244 | 0.20% |

**S4 Table** - Built models for each different target with their respective characteristics. Models' assessment was made using PROCHECK (within MODELLER) and the following MODELLER metrics: DOPE, GA341 score and molpdf. For all sequences, the best model was selected based on these metrics. DOPE (Discrete optimized protein energy) gives information by comparison of energies from different models generated taking into account the same sequence. It correlates with more native-like models, separating them from decoys. The lower its value, the better. GA341 scores, which consider the percentage sequence identity between the template and the model as a parameter, range from 0.0 (worst) to 1.0 (native-like). Molpdf (MODELLER's objective function) is a software's scoring function, representing the model quality, with lower values representing the best models. For each selected model, PROCHECK was employed to assess its stereochemical quality through the Ramachandran plots, assessing the percentage of residues in Ramachandran's allowed regions.

| Target | Fasta | Template | Align id (%) | Molpdf | DOPE | GA341 | Ramachandran core (%) |
|---|---|---|---|---|---|---|---|
| C | >137 | 6VG5 | 100.00 | 249.69 | -7566.60 | 1.00 | 97.10 |
| | >146 | | 98.75 | 247.44 | -7469.88 | 1.00 | 97.10 |
| | >158 | | 98.75 | 254.67 | -7375.54 | 1.00 | 98.50 |
| prM/M | >137 | 3C5X | 97.53 | 5.25E+02 | -7.62E+03 | 1.00 | 88.60 |
| | | 7BUD | 94.44 | 5.12E+02 | -6.15E+03 | 1.00 | 83.10 |
| | >145 | 7BUD | 93.06 | 4.28E+02 | -6.20E+03 | 1.00 | 86.20 |
| | >188 | 3C5X | 96.296 | 4.68E+02 | -7.64E+03 | 1 | 90 |
| | >190 | 7BUD | 95.833 | 5.11E+02 | -6.23E+03 | 0.99954 | 84.6 |
| | >207 | 7BUD | 93.056 | 4.43E+02 | -6.05E+03 | 0.99992 | 83.1 |
| E | >137 | 4UT6 | 98.99 | 2.26E+03 | -4.04E+04 | 1.00 | 91.40 |
| | >138 | | 98.48 | 2.39E+03 | -4.06E+04 | 1.00 | 92.90 |
| | >143 | | 98.73 | 2.30E+03 | -4.05E+04 | 1.00 | 91.70 |
| | >156 | | 98.22 | 2.30E+03 | -4.05E+04 | 1.00 | 91.70 |
| | >157 | | 98.73 | 2.41E+03 | -4.03E+04 | 1.00 | 92.00 |
| | >160 | | 98.73 | 2.30E+03 | -4.04E+04 | 1.00 | 90.50 |
| | >161 | | 98.73 | 2.30E+03 | -4.04E+04 | 1.00 | 90.50 |
| | >162 | | 98.48 | 2.28E+03 | -4.03E+04 | 1.00 | 91.70 |
| | >172 | | 98.48 | 2.31E+03 | -4.03E+04 | 1.0 | 91.40 |
| | >174 | | 98.22 | 2.60E+03 | -4.03E+04 | 1.00 | 91.70 |
| | > 188 | | 98.48 | 2.53E+03 | -4.04E+04 | 1.00 | 91.40 |
| | >190 | | 98.73 | 2.30E+03 | -4.04E+04 | 1.00 | 90.50 |
| | >191 | | 98.73 | 2.34E+03 | -4.02E+04 | 1.00 | 92.00 |
| | >202 | | 98.99 | 2.26E+03 | -4.04E+04 | 1.00 | 91.40 |
| NS1 | >137 | 4O6B | 96.87 | 2.96E+03 | -3.36E+04 | 1.00 | 88.70 |
| | >138 | | 96.55 | 2.96E+03 | -3.37E+04 | 1.00 | 89.60 |
| | >140 | | 96.87 | 3.07E+03 | -3.35E+04 | 1.00 | 85.10 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **>143** | | 96.55 | 2.89E+03 | -3.40E+04 | 1.00 | 89.00 |
| | **>145** | | 96.87 | 2.89E+03 | -3.38E+04 | 1.00 | 89.00 |
| | **>156** | | 96.55 | 2.84E+03 | -3.36E+04 | 1.00 | 88.70 |
| | **>158** | | 96.55 | 2.80E+03 | -3.34E+04 | 1.00 | 90.00 |
| | **>160** | | 96.87 | 2.98E+03 | -3.33E+04 | 1.00 | 88.70 |
| | **>169** | | 96.55 | 2.82E+03 | -3.32E+04 | 1.00 | 89.00 |
| | **>172** | | 96.55 | 2.95E+03 | -3.34E+04 | 1.00 | 90.00 |
| | **>173** | | 97.18 | 2.86E+03 | -3.33E+04 | 1.00 | 90.00 |
| | **>174** | | 96.87 | 2.97E+03 | -3.32E+04 | 1.00 | 88.70 |
| | **>179** | | 96.87 | 2.80E+03 | -3.31E+04 | 1.00 | 88.00 |
| | **>183** | | 96.55 | 2.91E+03 | -3.32E+04 | 1.00 | 90.30 |
| | **>201** | | 96.552 | 2.90E+03 | -3.35E+04 | 1 | 89.6 |
| | **>203** | | 96.552 | 2.93E+03 | -3.33E+04 | 1 | 89.3 |
| **NS3** | **>137** | **5YW1** | 78.54 | 4.06E+03 | -6.48E+04 | 1.00 | 91.20 |
| | **>138** | | 78.54 | 4.21E+03 | -6.48E+04 | 1.00 | 89.30 |
| | **>158** | | 78.54 | 4.24E+03 | -6.49E+04 | 1.00 | 90.50 |
| | **>160** | | 78.54 | 4.14E+03 | -6.46E+04 | 1.00 | 90.30 |
| | **>172** | | 78.37 | 4.32E+03 | -6.43E+04 | 1.00 | 90.30 |
| | **>180** | | 78.37 | 4.15E+03 | -6.51E+04 | 1.00 | 90.50 |
| | **>190** | | 78.54 | 4.23E+03 | -6.50E+04 | 1.00 | 90.50 |
| | **>194** | | 78.54 | 4.25E+03 | -6.45E+04 | 1.00 | 90.30 |
| | **>196** | | 78.37 | 4.10E+03 | -6.45E+04 | 1.00 | 89.50 |
| | **>201** | | 78.37 | 4.04E+03 | -6.50E+04 | 1.00 | 89.90 |
| | **>205** | | 78.54 | 4.17E+03 | -6.46E+04 | 1.00 | 89.70 |
| **NS5** | **>138** | **5ZQK** | 96.22 | 4.35E+03 | -1.03E+05 | 1.00 | 95.00 |
| | **>153** | | 96.00 | 4.31E+03 | -1.03E+05 | 1.00 | 95.40 |
| | **>147** | | 95.88 | 4.34E+03 | -1.02E+05 | 1.00 | 95.20 |
| | **>171** | | 96.22 | 4.46E+03 | -1.02E+05 | 1.00 | 95.10 |
| | **>137** | | 96.339 | 4.27E+03 | -1.03E+05 | 1.00 | 95.10 |
| | **>145** | | 96.11 | 4.54E+03 | -1.03E+05 | 1.00 | 95.60 |
| | **>168** | | 96.339 | 4.26E+03 | -1.03E+05 | 1.00 | 95.10 |
| | **>173** | | 96.224 | 4.29E+03 | -1.02E+05 | 1.00 | 95.00 |
| | **>140** | | 96.11 | 4.45E+03 | -1.02E+05 | 1.00 | 94.60 |
| | **>148** | | 95.767 | 4.59E+03 | -1.02E+05 | 1.00 | 95.10 |
| | **>151** | | 95.995 | 4.46E+03 | -1.02E+05 | 1.00 | 94.80 |
| | **>156** | | 96.224 | 4.47E+03 | -1.03E+05 | 1.00 | 95.00 |
| | **>157** | | 96.11 | 4.38E+03 | -1.02E+05 | 1.00 | 95.10 |
| | **>158** | | 96.11 | 4.50E+03 | -1.03E+05 | 1.00 | 95.10 |
| | **>160** | | 92.22 | 4.25E+03 | -9.59E+04 | 1.00 | 94.50 |
| | **>161** | | 94.165 | 4.31E+03 | -9.91E+04 | 1.00 | 95.40 |
| | **>162** | | 96.339 | 4.70E+03 | -1.02E+05 | 1.00 | 94.60 |
| | **>163** | | 96.11 | 4.23E+03 | -1.03E+05 | 1.00 | 95.50 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **>166** | 96.224 | 4.33E+03 | -1.02E+05 | 1.00 | 95.20 |
| | **>167** | 95.652 | 4.42E+03 | -1.01E+05 | 1.00 | 95.00 |
| | **>169** | 96.11 | 4.53E+03 | -1.02E+05 | 1.00 | 94.80 |
| | **>175** | 96.224 | 4.39E+03 | -1.02E+05 | 1.00 | 95.10 |
| | **>180** | 96.224 | 4.22E+03 | -1.02E+05 | 1.00 | 95.70 |
| | **>181** | 95.881 | 4.60E+03 | -1.02E+05 | 1.00 | 94.70 |
| | **>182** | 96.11 | 4.59E+03 | -1.02E+05 | 1.00 | 94.70 |
| | **>184** | 95.995 | 4.74E+03 | -1.02E+05 | 1.00 | 94.60 |
| | **>188** | 96.339 | 4.48E+03 | -1.02E+05 | 1.00 | 95.60 |
| | **>190** | 95.767 | 4.28E+03 | -1.01E+05 | 1.00 | 95.20 |
| | **>193** | 96.224 | 4.55E+03 | -1.02E+05 | 1.00 | 94.20 |
| | **>194** | 96.11 | 4.47E+03 | -1.02E+05 | 1.00 | 95.10 |
| | **>195** | 94.622 | 4.32E+03 | -9.87E+04 | 1.00 | 94.60 |
| | **>196** | 95.767 | 4.38E+03 | -1.01E+05 | 1.00 | 95.00 |
| | **>197** | 95.08 | 4.37E+03 | -1.00E+05 | 1.00 | 94.80 |
| | **>198** | 96.11 | 4.36E+03 | -1.02E+05 | 1.00 | 94.70 |
| | **>199** | 95.995 | 4.40E+03 | -1.02E+05 | 1.00 | 94.60 |
| | **>200** | 95.309 | 4.59E+03 | -9.91E+04 | 1.00 | 94.60 |
| | **>201** | 93.936 | 4.23E+03 | -9.91E+04 | 1.00 | 95.10 |
| | **>202** | 95.995 | 4.36E+03 | -1.03E+05 | 1.00 | 94.80 |
| | **>203** | 96.339 | 4.33E+03 | -1.03E+05 | 1.00 | 95.70 |
| | **>204** | 96.339 | 4.67E+03 | -1.03E+05 | 1.00 | 94.10 |
| | **>205** | 92.334 | 4.28E+03 | -9.58E+04 | 1.00 | 95.10 |
| | **>206** | 94.622 | 4.51E+03 | -9.87E+04 | 1.00 | 95.20 |
| | **>207** | 92.334 | 4.28E+03 | -9.68E+04 | 1.00 | 94.60 |
| | **>208** | 93.593 | 4.32E+03 | -9.64E+04 | 1.00 | 94.80 |

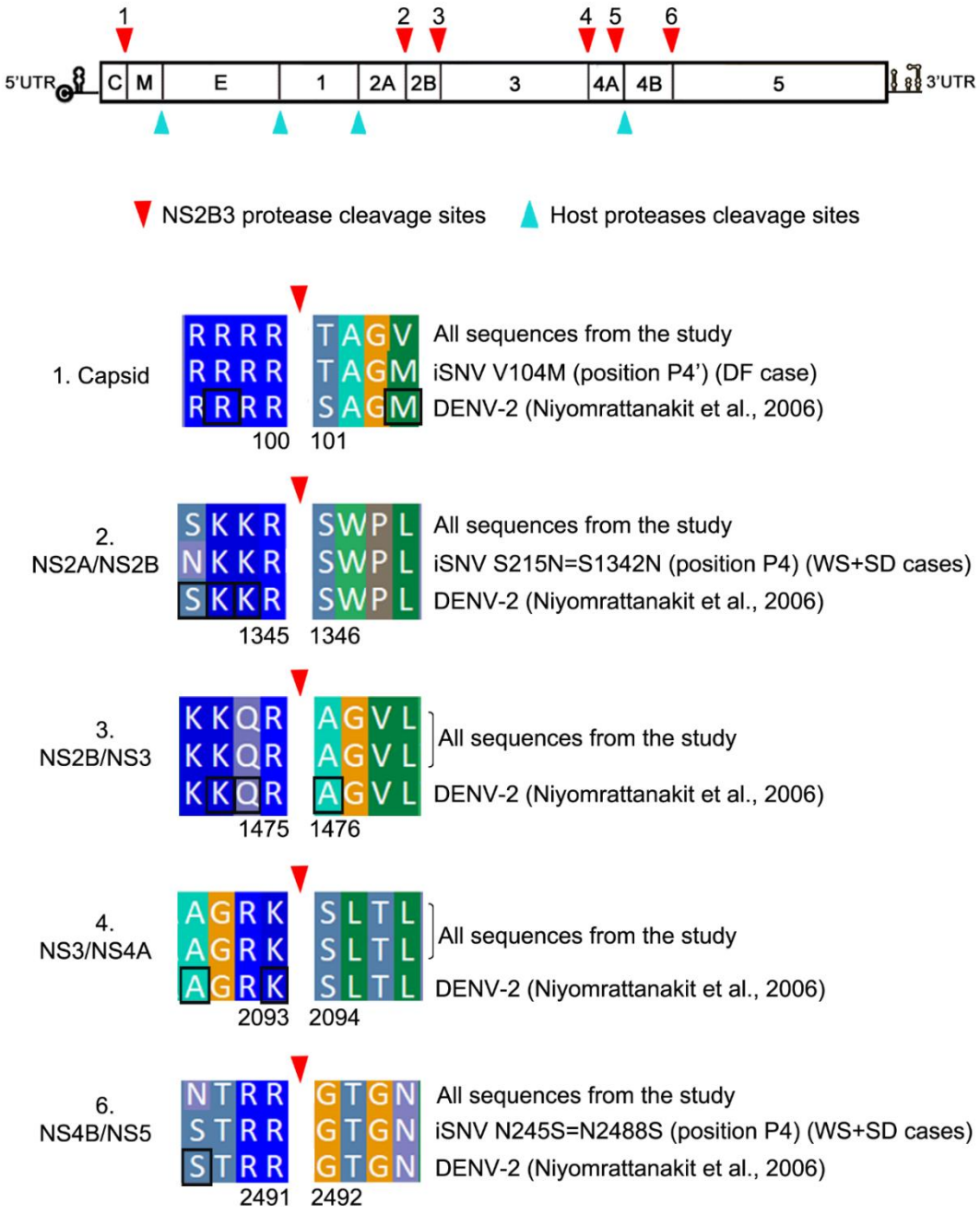
**Supplementary Table 5** - Thermodynamic disturbances caused by mutations located within UTRs' secondary structures. Predictions were made in RNAfold server. For each case, an optimal secondary structure with a minimum free energy (MFE) and and its thermodynamic ensemble (representing all the possible structures) were determined. As well, the following characteristics were estimated for each thermodynamic ensemble: the diversity and its free energy, the frequency of the MFE structure in the ensemble, and the centroid secondary structure with MFE, representing the structure with the minimum total base pair distance to all structures in the ensemble. SL: stem-loop; PK: pseudoknot; Hp: hairpin; DB: dumbbell-like; sHP: short hairpin; DF: dengue fever; WS: dengue with warning signs; SD: severe dengue; 2ry: secondary.

| | | Nucleotide Substitution | Status | DF cases [n (%)] | WS cases [n (%)] | SD cases [n (%)] | Samples' ID | Optimal 2ry structure MFE | Free energy of the thermodynamic ensemble | Frequency of MFE | Ensemble diversity | Centroid 2ry structure MFE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5UTR | SLA | SLA | | | | | 146-baseline | -22.3 | -22.83 | 42.36 | 2.24 | -22.3 |
| | | T10A | Consensus | 1(3.2) | - | - | 163 | -24.6 | -25.56 | 21.07 | 3.26 | -24.6 |
| | | G17A | Consensus | - | 1(5.3) | - | 157 | -22.2 | -22.73 | 42.44 | 2.13 | -22.2 |
| | | T12C+G17A+C21T+G22A | Consensus | 1(3.2) | - | - | 167 | -15.5 | -17.15 | 6.91 | 16.99 | -13.3 |
| | | T39C | Consensus | 2(6.5) | 3(15.8) | - | 153,154,159,187,192 | -18.1 | -18.84 | 30.09 | 4.28 | -18.1 |
| | SLB | SLB | | | | | 146-baseline | -7.4 | -7.69 | 62.47 | 0.67 | -7.4 |
| 3UTR | SLI | SLI | | | | | 137-baseline | -8.7 | -9.33 | 35.99 | 4.49 | -4 |
| | | G10313A | Consensus | 1(3.2) | - | - | 182 | -8.7 | -9.25 | 40.82 | 2.38 | -4 |
| | | T10326C | Consensus | 1(3.2) | - | - | 162 | -9 | -9.85 | 25.29 | 4.32 | -7.9 |
| | PK1-Hp | PK1-Hp | | | | | 137-baseline | -3.7 | -3.83 | 80.82 | 0.33 | -3.7 |
| | SLII | SLII | | | | | 137-baseline | -10.9 | -11.89 | 19.95 | 6.26 | -5.7 |
| | | T10386TinsCA | iSNV | - | - | 1(5.6) | 208 | -11.4 | -12.04 | 35.44 | 9.33 | -10.3 |
| | | C10387CinsAT | iSNV | - | - | 1(5.6) | 208 | -10.6 | -11.56 | 21.05 | 4.89 | -5.4 |
| | | C10387T | iSNV | - | - | 1(5.6) | 195 | -10.9 | -11.95 | 18.15 | 7.52 | -5.7 |
| | | A10388AinsC | iSNV | - | - | 1(5.6) | 208 | -12.2 | -12.61 | 51.14 | 6.2 | -12.2 |
| | | C10389T (+T10407C+10393insAA) | Consensus | 1(3.2) | 1(5.3) | - | 166,206 | -10.7 | -11.73 | 18.84 | 6.98 | -5.5 |
| | | C10389T | iSNV | 1(3.2) | 1(5.3) | 2(11.1) | 194,195 | -10.9 | -11.92 | 19.01 | 6.92 | -5.7 |
| | | | iSNV | | | | 191 | -8.9 | -9.99 | 16.98 | 8.22 | -5.3 |
| | | | iSNV | | | | 184 | -11 | -11.98 | 20.47 | 9.24 | -5.6 |
| | | C10389A (+T10407C+10393insA) | Consensus | 1(3.2) | 1(5.3) | - | 208 | -10.8 | -11.73 | 22.16 | 3.75 | -6.1 |

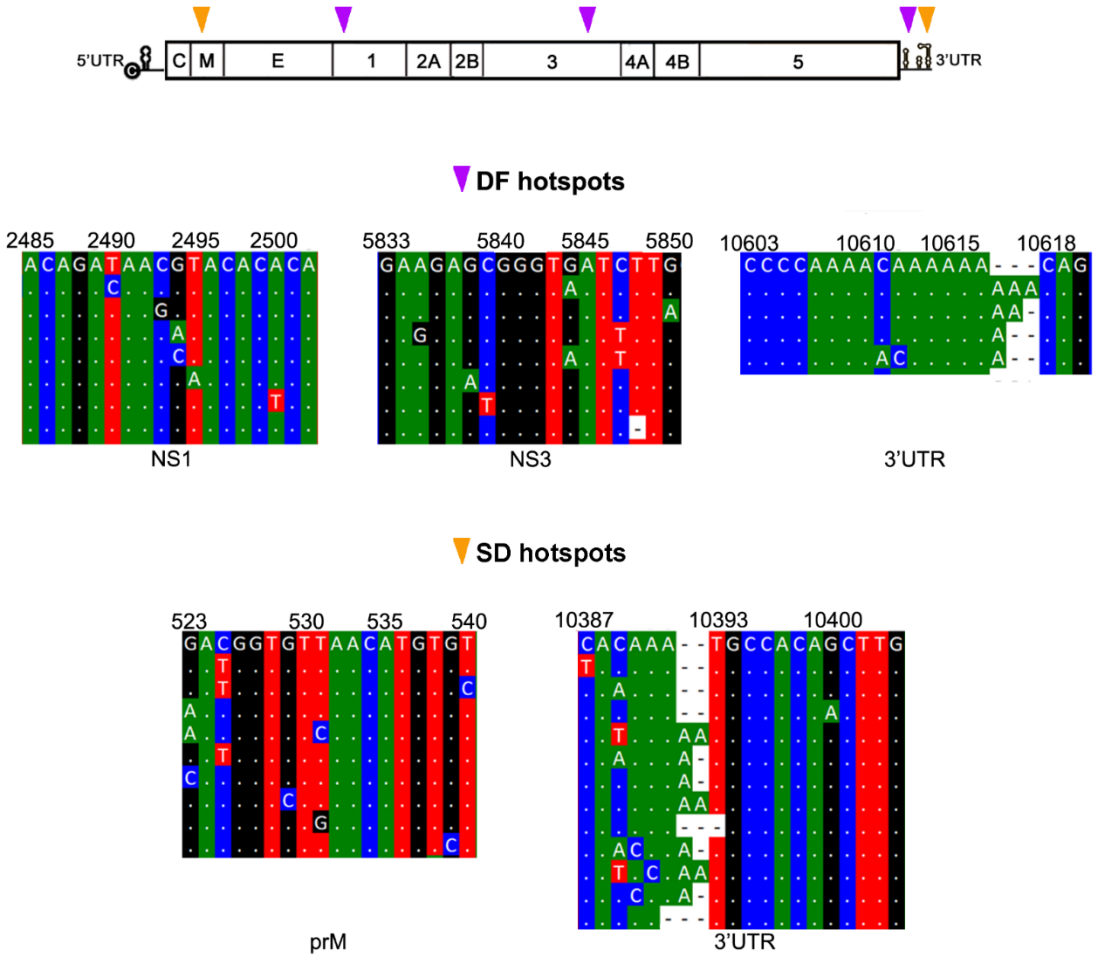
| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C10389CinsA | iSNV | 5(16.1) | 4(21.1) | 9(50.0) | 160,163,167,181,184,191,192,193,207,194,195,196,197,198,200,201,203,204 | -10.9 | -11.94 | 18.57 | 8.86 | -5.6 |
| | | C10389CinsAA | iSNV | - | 2(10.5) | 7(38.9) | 191,205,194,195,196,197,198,200,204 | -10.7 | -11.71 | 19.55 | 6.53 | -5.5 |
| | | 10390delA | iSNV | 9(29.0) | 2(10.5) | 2(11.1) | 160,161,162,163,167,169,181,182,184,190,193,209,201 | -12.3 | -12.76 | 47.66 | 6.61 | -12.3 |
| | | 10390delAA | iSNV | 5(16.1) | - | - | 161,162,168,170,173 | -12.3 | -12.76 | 47.66 | 6.61 | -12.3 |
| | | 10393delT | Consensus | - | 1(5.3) | - | 191 | -10.8 | -11.04 | 67.47 | 4.68 | -10.8 |
| | | 10393insA (+T10407C) | Consensus | 9(29.0) | - | - | 199,201,208,209,160,174,167,181,169,177,182,178,183,163,193,171,172,184 | -12.9 | -13.23 | 58.36 | 3.54 | -12.9 |
| | | 10393insAA (+T10407C) | Consensus | 22(71.0) | 1(5.3) | 2(11.1) | 173,161,175,168,162,170,166,179,180 | -12.2 | -12.61 | 51.14 | 6.2 | -12.2 |
| | | G10400A | iSNV | - | - | 2(11.1) | 195,200 | -12.3 | -12.61 | 60.66 | 1.18 | -12.3 |
| | | T10407C | Consensus | 22(71.0) | 4(21.1) | 7(38.9) | 140,157,160,161,162,163,166,167,168,169,170,171,172,173,174,175,177,178,179,180,181,183,184,190,193,194,195,198,201,206,208,209 | -12.3 | -12.76 | 47.66 | 6.61 | -12.3 |
| | | C10411T+G10413A | iSNV | - | - | 2(11.1) | 195 | -8.7 | -9.31 | 37.3 | 4.7 | -8.7 |
| | PK2-Hp | PK2-Hp | | | | | 137-baseline | -5.6 | -5.65 | 92.44 | 0.16 | -5.6 |
| | DB1 | DB1 | | | | | 137-baseline | -21.9 | -22.19 | 62.06 | 11.11 | -21.9 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A10463G | iSNV, Consensus | - | - | 2(11.1) | 195, 202 | -22.9 | -23.1 | 72.23 | 7.32 | -22.9 |
| | | C10520A | Consensus | 1(3.2) | - | - | 184 | -20 | -20.3 | 60.99 | 12.18 | -20 |
| | DB2 | DB2 | | | | | 137-baseline | -23.1 | -24.05 | 21.38 | 14.52 | -20.9 |
| | | C10540T | iSNV | - | - | 1(5.6) | 195 | -23.2 | -24.52 | 11.82 | 14.58 | -21 |
| | | A10563G | Consensus | 1(3.2) | - | - | 170 | -23.1 | -24 | 23.38 | 13.61 | -20.9 |
| | | T10566C | iSNV | - | - | 1(5.6) | 137 | -23.1 | -24.09 | 20.1 | 6.41 | -20.9 |
| | sHP | sHP | | | | | 137-baseline | -4.6 | -4.65 | 91.73 | 0.19 | -4.6 |
| | 3SL | 3SL | | | | | 137-baseline | -33 | -33.25 | 67.11 | 1.07 | -33 |
| | | C10660T | Consensus | - | - | 1(5.6) | 140 | -33.8 | -34.04 | 67.88 | 1.02 | -33.8 |
| | | C10684A | Consensus | - | 1(5.3) | - | 156 | -33 | -33.25 | 67.11 | 1.07 | -33 |
| | | C10687T | Consensus | - | - | 1(5.6) | 149 | -31.3 | -31.65 | 56.35 | 2.17 | -31.3 |
| | | A10715G+T10720A | Consensus | 1(3.2) | - | - | 162 | -44 | -44.06 | 90.94 | 0.25 | -44 |
| | | A10711T | iSNV | - | - | 1(5.6) | 149 | -32.6 | -32.82 | 69.88 | 1.62 | -32.6 |

**Supplementary Figure 6 -** Viral protease cleavage sites upon the polyprotein. Residues at positions P4 to P1 and P1' to P4' within each cleavage site are denoted in short alignments. Residues conserved among the four serotypes are highlighted with a black square. C: capsid; M: membrane precursor protein; E: envelope; UTR: untransated region; NS: non-structural; DF: dengue fever; WS: dengue with warning sign s; SD: severe dengue.



**Supplementary Figure 7 -** Mutational hotspots statistically supported. Nucleotide alignments of each hotspot show all different iSNV/SNP involved in the mutational burden within the 18-nucleotide window. C: capsid; M: membrane precursor protein; E: envelope; UTR: untransated region; DF: dengue fever; SD: severe dengue.



## 5. DISCUSSÃO

No Brasil, a transmissão do DENV-2 tem ocorrido de forma praticamente continuada desde a sua introdução em 1990, com ondas epidêmicas intercaladas com períodos de silêncio epidemiológico, consistente com possíveis ciclos epizoóticos na natureza e introduções de novas cepas no país (Faria et al., 2013; Figuereido, 2019; SVS/MS, 2019). A reintrodução deste sorotipo no ano 2007 envolveu uma cepa diferente às que previamente tinham circulado no país, definida como linhagem BR3, e pertencente a uma linhagem superior chamada de clado IV por Mir e colaboradores (Mir et al., 2014). Não só se evidenciou uma grande distância filogenética entre esta última e a linhagem BR1, pertencente ao clado III e responsável pela introdução do DENV-2 no Brasil, senão que também houve uma mudança no padrão clinico-epidemiológico da doença, com um nítido aumento da gravidade (Nunes et al., 2016). Depois de três anos de grandes epidemias causadas pela linhagem BR3, a circulação de DENV-2 foi praticamente substituída pelos sorotipos DENV-4 e DENV-1, assim como posteriormente por outros arbovírus como ZIKV (Zika vírus), YFV (vírus da Febre Amarela) e CHIKV (Chikungunya vírus) (SVS/MS, 2012-2017). No entanto, durante os anos 2017 e 2018 que sucederam à epidemia de ZIKV, o DENV-2 se fez visível novamente, espalhando-se pelas regiões centro-oeste e sudeste principalmente. Cabe destacar que durante estes anos as notificações de casos de DENV foram surpreendentemente baixas (SVS/MS, 2019c). Ao respeito, especula-se que este fenômeno possa estar relacionado com a proteção cruzada transitória da exposição ao ZIKV, ao invés de mudanças nas condições ecológicas ou lacunas na vigilância (Borchering et al., 2019; Fernandes Brito et al., 2020). Porém, já em 2019, houve um importante ressurgimento deste sorotipo, tornando-se o responsável da notificação de 1,68 milhões de casos no país (80% dos casos totais de DENV), com uma incidência de 1078,5 casos por 100.000 habitantes e uma taxa de letalidade de 0,04, envolvendo principalmente idosos maiores de 60 anos (SVS/MS, 2020; PAHO, 2020). Embora tenha-se descrito um aumento absoluto de casos e óbitos em 2019, a epidemia de DENV-2 dominando principalmente as regiões centro-oeste e sudoeste do país, não foi associada a um aumento significativo na taxa de letalidade em comparação com anos anteriores (PAHO, 2020).

Em consequência da exacerbada circulação de DENV-2 durante o ano 2019, diversos estudos foram desenvolvidos com o intuito de descrever qual a origem desta cepa e o seu possível impacto epidemiológico, considerando que a transmissão deste sorotipo no período 2012-2018 tinha sido praticamente silenciosa. Desta maneira, a análise apresentada no artigo 1, pioneira dentre todos os estudos, demonstrou que a cepa detectada no estado do Rio de Janeiro pertencia ainda ao clado IV contendo a linhagem que causou o surto em 2008, no entanto, apresentava um agrupamento filogenético num cluster totalmente separado das sequências previamente obtidas durante o surto anterior, e conjuntamente com sequências de Porto Rico. Segundo a análise desenvolvida com a amostragem descrita, este país teria dado origem ao ancestral da cepa circulante no Brasil em 2019. Resulta importante notar que até o início de 2019, nenhum caso de DENV-2 tinha sido diagnosticado nas ilhas do Caribe (PAHO, 2019), o que sugeria claramente que a cepa de 2019 do Rio de Janeiro poderia ter circulado em outro lugar, até sua chegada ao estado, ou poderia ter se replicado silenciosamente por algum tempo até atingir um nível detectável. Era provável que, na verdade, a cepa viral que entrou no estado do Rio de Janeiro tivesse sido introduzida primeiro em um estado brasileiro diferente e ter-se espalhado então para o Rio de Janeiro, com base nos casos crescentes de DENV-2 relatados em outros estados.

Estudos posteriores ao nosso chamaram a esta nova cepa de linhagem BR4 e confirmaram uma possível origem da mesma no caribe (De Jesus et al., 2020, Adelino et al., 2021), embora haja uma grande lacuna temporal entre a amostragem das sequências caribenhas de 2005 e as sequências brasileiras deste último surto, conforme discutido anteriormente no artigo e nestes novos estudos (De Jesus et al., 2020, Adelino et al., 2021). Por outro lado, também foram identificados isolados pertencentes à linhagem BR3 responsáveis de casos de DENV-2 durante os últimos cinco anos, o que demostra que existe uma co-circulação das duas linhagens, mesmo que com predominância da BR4. Pela sua vez, Brito e colaboradores determinaram que o recente surto de dengue foi causado por uma linhagem de DENV-2 que circulava no Brasil antes da epidemia de Zika, o que significa que a mesma estabeleceu uma transmissão críptica por aproximadamente cinco anos antes de causar o surto em 2019, talvez devido ao declínio da proteção

cruzada fornecida pela imunidade populacional existente para ZIKV (Fernandes Brito et al., 2020).

Estes estudos que sucederam ao exibido no artigo 1, permitiram superar algumas das limitações concomitantes do nosso, por terem empregado amostragens maiores, e mais representativas em alguns casos, assim como também por terem utilizado sequencias do genoma viral completo. Paralelamente, o fato de serem posteriores temporalmente, permitiu uma análise de dados maior e mais atualizada. Desta forma, a menção feita no artigo 1 acerca de que os casos de 2019 do Rio de Janeiro apresentaram características clínico-epidemiológicas diferentes em relação aos de 2008, perde validade quando se têm em conta dados atualizados do país inteiro. Conforme citado anteriormente, a taxa de letalidade deste surto no nível nacional não foi menor que a de anos anteriores, a as faixas etárias mais acometidas nos casos fatais foram principalmente os idosos maiores de 60 anos, seguidos das crianças menores de 9 anos (SVS, 2020). Cabe destacar que os dados analisados do primeiro semestre de 2019 no estado do Rio de Janeiro possam também estar influenciados pelo surto de chikungunya de grande magnitude que assolou ao estado (SVS/MS, 2018). É provável que a competência do vetor por ambos os vírus tenha levado a uma circulação mais baixa de DENV-2 e, portanto, a um número reduzido de casos. Neste cenário, as probabilidades de terem se detectado casos com maior gravidade eram bastante reduzidas.

Pelo exposto acima, é que se considerou que embora a BR4 possa ser uma nova linhagem se espalhando pelo Brasil, a mesma não resulta tão distante geneticamente da BR3, a diferença do observado entre BR1-BR3. Além disso, ao contrário do que foi descrito para estas últimas (Nunes et al., 2016), não se destacaram nítidas diferenças no padrão clínico-epidemiológico entre BR3 e BR4. Assim, os isolados pertencentes à linhagem BR4 foram incluídos na amostragem das análises subsequentes de diversidade intra-hospedeiro de DENV-2.

Conforme comentado anteriormente, além de ser um país hiper endêmico para DENV, o Brasil tem enfrentando também surtos dos vírus Zika e da febre amarela (SVS 2017a-b), para os quais a reatividade cruzada com o DENV continua sendo um tópico de discussão entre os cientistas (Bradina et al., 2017; Keasey et al., 2017). Por outro lado, foi lançada ao mercado a vacina contra DENV “Dengvaxia”, sendo somente administrada na rede privada de saúde. Previas exposições a outros Flavivírus ou a imunização com a vacina “Dengvaxia” podem

atuar como fatores de seleção e resultam de alto potencial no surgimento de novas cepas virais. Já foi proposto que, devido às pressões de seleção convergentes, novos “hotspots” de diversidade no genoma viral podem surgir dentro de cada hospedeiro (Parameswaran et al. 2017). Sim e colaboradores demonstraram que as populações virais são capazes de restaurar rapidamente sua diversidade após um evento de restrição, criando predominantemente um repertório de SNVs muito diferente que provavelmente se origina de mutações aleatórias (Sim et al., 2015). Por outro lado, foi evidenciado em diferentes experimentos com vírus RNA, que somente uma ou poucas substituições de amino ácidos em uma única proteína bastam para modificar as características biológicas de um vírus (Tsetsarkin et al., 2007, Pfeiffer et al., 2003, Pompon et al., 2017). E assim como outros vírus de RNA, o DENV-2 não é a exceção, e continua a evoluir. Pressões de seleção como as acima nomeadas poderiam forçar o surgimento de mutações adaptativas que só sejam identificadas quando se tornem dominantes na população viral, um processo que pode levar meses ou anos.

Além disso, diversas investigações mostraram evidências de uma forte associação entre a composição genética de populações virais intra-hospedeiro e aptidão viral e patogenicidade (Sullivan et al., 2007; Lee et al., 2008; Fitzsimmons et al., 2018; Vignuzzi et al., 2019), especialmente nos últimos anos em que técnicas de sequenciamento de nova geração com cobertura alta e acurada do genoma viral completo, forneceram provas de que as variações de aptidão e adaptabilidade ocorrem sem mudanças na sequência consenso viral (Parameswaran et al., 2012; Rodriguez-Roche et al., 2016; Parameswaran et al., 2017). No entanto, conforme recentemente revisado por Ko e colaboradores, apenas uns poucos estudos rastrearam a diversidade intra-hospedeiro de DENV em contextos epidemiológicos (Parameswaran et al., 2012; Rodriguez-Roche et al., 2016; Parameswaran et al., 2017; Ko et al., 2020). Todos eles combinaram estratégias experimentais de amplificação do genoma viral por PCR + sequenciamento profundo. Ao contrário deles, este estudo empregou uma abordagem de sequenciamento profundo sem a necessidade de criar amplicons, com o objetivo de refletir de forma fiel a diversidade genética viral em cada estágio da infecção dos pacientes, para finalmente avaliar a relação da mesma com a gravidade da doença. Manter essa fidelidade e obter as “fotos instantâneas” do paciente para analisar a diversidade intra-hospedeiro do DENV-2 significou renunciar a alta profundidade de cobertura do sequenciamento

para amostras com baixa carga viral. Os casos de dengue grave que de maneira geral apresentaram baixas cargas virais, são um ponto chave deste estudo. Normalizar a carga inicial de RNA de acordo com a amostra limitante levaria a uma importante perda de informações nas amostras restantes. Sendo assim, para evitar vieses e ao mesmo tempo manter a representação do real cenário intra-hospedeiro do DENV-2 em cada paciente, aplicamos vários filtros nos passos finais da chamada de variantes para reduzir potenciais erros, mas não se excluíram amostras de baixa carga viral inicialmente incluídas no estudo (apesar da variação na profundidade obtida no sequenciamento). Desta maneira, os 68 casos sob estudo foram clinicamente agrupados e padrões que pudessem emergir da análise da diversidade intra-hospedeiro para cada categoria foram procurados.

Nos RNA vírus, a geração de variabilidade é determinada pela natureza da RNA polimerase propensa a erros e a sua taxa de replicação, enquanto a prevalência de mutantes individuais depende apenas do *fitness* daquele mutante particular (Andino & Domingo, 2016). Neste estudo, conforme descrito no artigo 2, se observou que os casos de DF apresentaram maiores proporções de variantes não sinônimas e indels (chamadas no artigo de NS-iSNV + iLVs, do inglês “intrahost single nucleotide variant” e “intrahost long variant”) do que WS e SD, aumentando assim a chance de mutações não sinônimas prejudiciais, proteínas truncadas e partículas virais defeituosas (Figura 2C, artigo 2). Duas explicações possíveis devem ser consideradas ao respeito: 1) neste estado genético altamente dinâmico nos casos de DF, a progressão da doença para a gravidade pode não ser facilitada, ou 2) assim como sugerido por Gregory e colaboradores, a diversidade genética viral mais rica presente nos hospedeiros DF poderia ser benéfica, pois concederia à população viral uma maior capacidade de adaptação às mudanças ambientais na medida que a doença evolui (Gregori et al., 2016). No entanto, considerando também as que de maneira geral, as frequências das variantes intra-hospedeiro foram menores nestes casos (Figura 2B, artigo 2), e o fato desses casos apresentarem tempos de evolução mais curtos do que WS e SD no processo da infecção, a explicação mais provável seria que as diferenças observadas entre os grupos clínicos possam ser devido a distintos tempos de evolução intra-hospedeiro. Recentemente, foi demonstrado o processo mediante o qual a pressão imunológica intra-hospedeiro molda a diversidade do DENV-3 durante as infecções humanas, e como esse processo pode ser afetado pelo histórico imunológico do paciente

(Rodriguez-Roche et al., 2016; Parameswaran et al., 2017). Consistentemente, isso explicaria porque os casos de SD, submetidos a um processo de seleção purificadora prolongado e exaustivo (amostras coletadas a partir do quarto dia desde o início dos sintomas, em diante), acumularam uma proporção maior de SS-iSNVs (Figura 2C, artigo 2).

Ao analisar como a variabilidade se comportou ao longo do genoma viral, observou-se que apesar das pequenas diferenças vistas para três dos genes não estruturais (NS2A, NS2B e NS4A) entre as três categorias clínicas, uma tendência comum se destacou para o gene NS2B, no que diz respeito a sua baixa proporção de NS-iSNVs, independentemente da clínica dos pacientes (Figura 2C, artigo 2). Isto pode ser interpretado como uma atividade da proteína mutada mal suportada pela população viral no geral. Nesse caso, seria esperado que as NS-iSNVs detectadas fossem apenas conservadoras, ou variantes de evolução recente, que ainda não tenham sido selecionadas a favor ou em contra. NS2B é um cofator da protease NS3, necessário para a clivagem da poliproteína. Para uma atividade enzimática completa, NS3 requer a interação com o loop estrutural de NS2B que compreende os resíduos 49 a 95 (León-Juárez et al., 2016). Curiosamente, Parameswaran e colaboradores observaram também uma menor diversidade intra-hospedeiro neste gene, quando comparado aos restantes (Parameswaran et al., 2012). Estas evidencias fazem deste gene um candidato ou alvo interessante para o desenho de drogas e/ou vacinas.

Ao contrário do observado em infecções secundárias por DENV-3 (Rodriguez-Roche et al., 2016), não encontramos diferenças na variabilidade entre os casos primários e secundários desta coorte. No entanto, as infecções secundárias mostraram uma proporção maior de NS-iSNVs do que as primárias (teste T-student pareado, p= 0.0132), consistente com seus tempos de infecção mais curtos (62% apresentaram menos de 3 dias de sintomas, em comparação ao 38,5% entre os casos primários) e, provavelmente, com as respostas dirigidas. Em infecções secundárias, os anticorpos heterólogos produzidos durante as infecções primárias poderiam estar agindo assim que o vírus entrasse no hospedeiro, depurando rapidamente as partículas virais defeituosas, produtos de variantes de inserção/deleção no genoma viral, o que seria consistente com a maior proporção de iLVs encontrada entre os casos primários. Além disso, a pressão seletiva imposta poderia ser a causa das discrepâncias observadas para a proporção de

NS-iSNV em prM/M e NS1, dois genes codificantes altamente imunogênicos. Notavelmente, ao contrário de outros estudos, não se detectou diferença alguma entre a diversidade intra-hospedeiro do gene E dos casos primários e secundários (Parameswaran et al., 2012; Rodriguez-Roche et al., 2016; Parameswaran et al., 2017).

Por outro lado, se os anticorpos heterólogos facilitassem a replicação do vírus, como afirmava a teoria do ADE, e, portanto, a diversidade genética, seria de se esperar que os casos secundários apresentassem carga viral maior do que os primários. Porém, nenhuma diferença significativa foi detectada entre as suas medianas. Embora possa parecer contrário aos achados comuns, nos quais os casos secundários tendem a desenvolver quadros clínicos mais graves devido ao efeito ADE, é importante considerar que Katzelnick e colaboradores descreveram uma janela especifica no título de anticorpos heterólogos preexistentes correlacionada com o aumento da gravidade da infecção (Katzelnick et al., 2017). É provável que alguns casos secundários desta coorte tivessem títulos de anticorpos pré-existentes fora da janela e, portanto, evitassem a infecção grave. Na verdade, apenas quatro dos casos secundários desenvolveram SD, sendo que três apresentaram os títulos de anticorpos mais elevados (Tabela S7, artigo 2). De qualquer forma, as conclusões tiradas da análise dos casos agrupados por resposta imune devem ser cuidadosas, uma vez que nenhuma correlação estatística foi detectada nesta coorte entre a resposta imune dos pacientes e o resultado clínico.

As iSNVs não sinônimas e aquelas detectadas nas UTRs (NS-iSNVs + UTRs-iSNVs) dos casos de SD poderiam ser consideradas como as mutantes que melhor se adaptaram ao ambiente do hospedeiro e à pressão seletiva subsequente durante os 4 ou mais dias do curso da doença. As UTRs apresentam domínios altamente estruturados fundamentais para a replicação viral, e que pela sua vez ajudam ao vírus a escapar da resposta imune inata do hospedeiro, contribuindo assim ao desfecho clínico no hospedeiro (revisado em Ng et al., 2017). Por serem áreas tão importantes, consideramos fundamental avaliar também a presença de mutações nelas. Portanto, e para determinar se havia algum padrão mutacional característico nas amostras agrupadas pelas categorias clínicas, NS-iSNVs+UTRs-iSNVs consistentemente encontradas entre as amostras foram classificadas de acordo com sua presença em cada grupo (Figura 3, artigo 2).

O primeiro subconjunto de variantes repetitivas foi encontrado

exclusivamente entre os casos de DF e em baixas frequências intra-hospedeiro (subgrupo 1, Tabela S5), sugerindo que essas variantes poderiam não estar envolvidas na patogênese grave do DENV-2. Porém, não está claro se elas poderiam representar algum prejuízo para o vírus, induzindo assim um baixo *fitness* e, levando em última instância à extinção viral. No entanto, isto poderia ser uma possível explicação para a sua baixa frequência (também devido aos tempos de infecção mais curtos) e a falta da progressão dos casos de DF para a gravidade.

Dentre os subgrupos 2 e 3, compostos por variantes encontradas apenas nos casos WS+SD, 5 e 4 NS-iSNVs repetitivas foram detectados numa primeira instância como variantes relevantes potencialmente correlacionadas com SD (Figura 3, artigo 2), respectivamente, devido tanto às suas frequências inter e intra-hospedeiro, como ao seu possível efeito nas proteínas virais. No entanto, a sua presença no nível consenso em amostras diferentes desses portadores e pertencentes também aos casos de DF, descartou essa hipótese anterior. Por outro lado, esta observação pode representar um indício de que algumas subpopulações virais podem ser transmitidas de forma eficaz após a superação da barreira humano-mosquito. Apoiando nossa linha de evidência, estudos anteriores com vários modelos virais comprovaram a transmissão efetiva de subpopulações, demonstrando que os espectros de mutantes das populações virais possuem genomas minoritários que refletem aqueles que foram dominantes em fases anteriores da sua evolução, servindo como um reservatório molecular que é capaz de reagir rapidamente ante restrições seletivas que foram experimentadas anteriormente pela mesma população (Ruiz-Jarabo et al., 2000; Briones et al., 2006; Park et al., 2015). Deste ponto de vista, surge a pergunta acerca de se seria possível que essas variantes fossem apenas relevantes para a transmissão, mas não para a patogênese, considerando também que foram achadas em amostras de soro. Ainda nestes dois subgrupos, dois UTR-iSNVs envolvendo inserções de 1–2 nucleotídeos nas posições 10389 e 10611 foram destacadas como possíveis relevantes (setas coloridas na Figura 3, artigo 2). Enquanto 10611insA não comprometeria nenhuma estrutura secundária de RNA, 10389insAA está localizada dentro do “Stem-loop I”, uma estrutura de RNA envolvida na geração e acumulação de RNAs de flavivírus subgenômicos, que desempenham papéis essenciais neutralizando as respostas antivirais no mosquito e nas células humanas (Moon et al., 2015). O possível efeito desta última será discutido mais tarde.

Finalmente, de particular interesse foram as variantes minoritárias detectadas entre os casos pertencentes aos três grupos clínicos (subgrupo 4; Tabela S5, artigo 2). Elas podem ser pensadas como o reservatório de variabilidade capaz de vencer os gargalos de transmissão entre humanos e mosquitos, que quando expostos a uma forte seleção purificadora (como nos casos de SD), podem evoluir restaurando os espectros de mutantes. É provável que variantes repetitivas desse subgrupo estejam refletindo esse cenário. Por sua vez, 5 das mais relevantes foram detectados em nível de consenso em até três casos de WS+SD.

Embora o foco tenha estado sobre as variantes repetitivas, as únicas, ou seja, aquelas presentes em apenas uma amostra só, não devem ser deixadas de lado. Mesmo sendo impossível traçar um padrão mutacional a partir delas, sua participação na patogênese, seja pelo seu próprio potencial ou por possíveis efeitos epistáticos, não pode ser descartada. Em relação a estes últimos, também vale a pena investigar no futuro se o efeito combinado de NS-iSNVs repetitivas detectadas nos casos de SD poderia desencadear fenótipos graves. Relações epistáticas foram recentemente documentadas para DENV (Bellone et al., 2020; Syenina et al., 2020).

Por outro lado, embora este estudo não tenha apontado principalmente ao repertório de iSNV sinônimas devido à sua natureza, deveria dar-se uma atenção especial no futuro próximo, pois há muitas evidências mostrando que não necessariamente causam mudanças neutras (Novella et al., 2004; Nougairede et al., 2013). Embora as mutações sinônimas sejam frequentemente consideradas seletivamente neutras, a variação observada no uso de códons nos diferentes modelos virais sugere a presença de um viés mutacional e/ou de uma pressão seletiva (Jenkins e Holmes, 2003; Plotkin e Kudla, 2011). Como várias opções de códons estão disponíveis para um determinado aminoácido, existe comumente uma seleção fraca para códons sinônimos que são usados com maior frequência, neste caso, corresponderia aos códons humanos mais usados. Acredita-se que a seleção de códons frequentes ocorra principalmente porque os códons comuns são traduzidos mais rapidamente (fornecendo um maior controle regulatório) e com maior fidelidade (produzindo sequências de proteínas mais precisas) do que os códons raros. Assim, genes altamente expressos serão, portanto, enriquecidos com códons comuns (Clarke & Clark, 2008). Uma vez que a maior influência do uso de códons é na taxa de tradução local, pode-se pensar que a replicação viral poderia

se adaptar ao uso dos códons humanos, otimizando a sua taxa de tradução com um impacto potencial na expressão da proteína e no *fitness* viral. Um estudo desenvolvido com Poliovírus geneticamente modificado com centenas de mutações sinônimas demostrou que as populações virais resultantes exibiam espectros de mutantes únicos. Fenotipicamente tinham capacidade replicativa semelhante, porém diferiam no *fitness*, sendo uma delas menos mutacionalmente robusta do que as outras, e surpreendentemente, também mais atenuada em um modelo de infecção animal, demonstrando a importância dos espectros de mutantes como o todo na determinação da patogênese viral (Lauring et al., 2012).

De maneira geral, os resultados apresentados no segundo eixo de execução deste projeto mostraram que a estrutura da população viral intra-hospedeiro das amostras estudadas variou de acordo com a gravidade da doença DENV-2, em decorrência direta do tempo de infecção, contribuindo para o entendimento da dinâmica viral desse vírus. Cabe destacar que, uma das limitantes deste trabalho resulta do fato de tratar-se de um estudo de tipo transversal e retrospectivo, no qual se obtiveram “fotos instantâneas” dos diferentes pacientes em diferentes estágios do curso da infeção por DENV. Idealmente, para poder obter uma imagem mais abrangente do cenário real que envolve a dinâmica da infecção por DENV em um hospedeiro humano a traves do tempo, deveria desenvolver-se um estudo de tipo longitudinal, que avaliasse a diversidade viral periodicamente ao longo do tempo dentro de um mesmo paciente. Desta maneira, a relação entre a diversidade viral intra-hospedeiro com a patogênese viral e a apresentação clínica da infecção, poderia ser evidenciada mais claramente.

O próximo passo resultava então determinar se efetivamente existia um significado estrutural ou funcional para as mutações consideradas relevantes aqui relatadas. Desta forma, ensaios por modelagem molecular foram desenvolvidos com o objetivo de nos aproximar o máximo possível à identificação de potenciais variantes virais relevantes para a dinâmica da infecção por DENV-2.

Um conjunto de 141 mutações que incluiu iSNVs e SNPs, tanto não sinônimas localizadas ao longo da poliproteína quanto outras localizadas nas UTRs, foi definido conforme descrito na seção anterior, e estas foram avaliadas sobre os modelos criados das proteínas virais e das estruturas secundárias típicas das UTRs do RNA viral, conforme descrito no artigo 3.

Abordagens de modelagem molecular foram empregadas para construir

modelos 3D para diferentes proteínas virais. Para as proteínas estruturais (C, prM e E) e três proteínas não estruturais (NS1, NS3 e NS5), uma estratégia de modelagem comparativa foi implementada uma vez que estruturas obtidas de experimentos de cristalografia de raios-X para esses alvos estavam disponíveis em bancos de dados abertos. No entanto, para as proteínas virais NS2A, NS2B, NS4A e NS4B, foram necessárias diferentes abordagens, pois todas elas são proteínas inseridas na membrana do retículo endoplasmático (RE), e ninguém foi capaz de modelá-los adequadamente ainda hoje. Diferentes estratégias têm sido propostas para resolver as suas topologias empregando ensaios bioquímicos e/ou ressonância magnética nuclear, os quais foram cruciais para obter fragmentos das respetivas estruturas que permitissem em seguida montar o quebra-cabeças da topologia mais provável (figura 8, artigo 3). Consequentemente, torna-se evidente que nenhum modelo cristalográfico disponível poderia servir como ponto de partida para qualquer estratégia clássica de modelagem por homologia. Assim, neste trabalho, empregamos estratégias de reconhecimento de enovelamento e “de novo” para construir modelos que nos permitissem avaliar o efeito das mutações que se localizavam nessas proteínas. Apesar de várias tentativas, mesmo considerando as coordenadas das topologias existentes como informações de entrada para a modelagem, as estruturas construídas não atenderam aos parâmetros de qualidade exigidos para um modelo aceitável. Cabe destacar que modelar proteínas inseridas em membrana ainda é um desafio significativo na área. Programas amplamente reconhecidos na literatura e comumente usados para a modelagem 3D de proteínas ainda encontram esse como um problema desafiador a resolver. Neste tipo de análise seria importante considerar a membrana em estudos de refinamento do modelo por dinâmica molecular, o que resulta computacionalmente caro por causa do elevado número de parâmetros termodinâmicos a serem verificados, e demorados pois se precisa muito tempo para observar o enovelamento da membrana e a correta parametrização dos campos de força envolvidos. Consequentemente, considerando que os modelos obtidos não garantiam a precisão das análises, as mutações foram julgadas apenas considerando as topologias propostas e as informações coletadas para cada região proteica.

Coletivamente, todos os resultados expostos no artigo 3 ao respeito das mutações avaliadas nos modelos 3D efetivamente construídos mostraram que, em linhas gerais, as variantes disruptivas foram identificadas principalmente entre os

casos de DF. Dentre elas, por exemplo, podem citar-se L50F e R82I localizadas na proteína do capsídeo, envolvidas numa possível atenuação na produção de partículas virais e na interação com o RNA viral, respetivamente, N7H no precursor da proteína de membrana prM, possivelmente alterando a interação entre prM e a proteína do envelope, L153M e E326G em NS1, com potenciais alterações da replicação viral e da interação NS1-capsideo, respetivamente, assim como também C261W, A263D e R377I em NS3, envolvidas as duas primeiras na interação entre o motivo Ic do domínio helicase e o RNA fita simples, enquanto a última causa uma mudança na carga do arranjo DEERE na superfície da proteína, com uma potencial diminuição nas atividades ATPase e helicase. Pelo contrário, potenciais variantes de escape imunológico foram associadas principalmente aos casos de WS+SD, o que faz sentido com os tempos de evolução intra-hospedeiro mais longos destes últimos. Estas últimas se localizaram na proteína precursora de membrana prM (G15S, D29N e I39M), na proteína do envelope (E71A, D154N e G330D, nos domínios II, I e II, respectivamente) e em NS1 (T126I e D290N), a qual embora seja uma proteína não estrutural, a sua forma hexamérica é secretada da célula infectada atuando como um potente imunógeno (Akey et al., 2014). Os resíduos envolvidos foram já definidos como parte de epítopos ou dentro de possíveis regiões de reconhecimento antigênico por anticorpos e outros componentes do sistema imunológico (Vazquez et al., 2002; Akey et al., 2014; Luo et al., 2015; Rouvinsky et al., 2015; Qiu et al., 2018). Cabe destacar que dentre as 141 mutações analisadas não foi achada nenhuma que aparente estar relacionada com uma maior virulência ou patogenicidade. As evidências sugerem ao contrário, que as mutações identificadas entre os casos de WS+SD seriam o resultado da superação dos gargalos seletivos impostos pelas próprias barreiras do hospedeiro humano. Resultou chamativo, porém, que muitas das variantes aqui estudadas se localizaram em resíduos da superfície das respetivas proteínas, os quais poderiam estar envolvidos em interações proteína-proteína ainda desconhecidas. Um exemplo claro é o caso de NS5, na qual 18 das 27 mutações ocuparam posições superficiais tanto no domínio metiltransferase quanto no polimerase dependente de RNA. Vários pontos de contato foram descritos entre NS5 e NS3, com o SLA em 3'UTR, ou na proteína β-importina da célula hospedeira (Brooks et al., 2002; Iglesias et al., 2011), mas nenhum deles se mostrou comprometido por essas mutações. No entanto, a NS5 interage com muitas outras proteínas do hospedeiro

relacionadas à montagem do fuso, splicing, modificações da cromatina e imunomodulação, entre outras (Fernandez-Garcia et al., 2011; Shah et al., 2018), sobre as quais ainda não se sabe como é que acontece essa interação. Consequentemente, o papel dessas mutações não deve ser descartado, embora possam parecer neutras para o funcionamento eficiente do NS5. Pela sua vez, três variantes presentes nos casos de DF foram identificadas nas proteínas NS4A (Y41H e A63T) e NS4B (N245S), particularmente em regiões presumíveis de interação tanto com NS1 e NS4B, no caso da primeira, quanto com NS5 no caso da última. Porém, deve ressaltar-se que estas últimas apenas foram identificadas a partir das topologias propostas e não de modelos construídos das proteínas em questão. Novos estudos serão indefectivelmente necessários para desvendar as complexas interações entre as proteínas virais e do hospedeiro, uma vez que há uma tendência crescente de estudos relatando interações com proteínas do hospedeiro, críticas para tanto para processos de imunomodulação quanto para o desvio no uso da maquinaria celular para aproveitamento viral.

Embora amplamente aceitas, as análises “in-silico” apresentam algumas limitações e, em hipótese alguma, substituem as do tipo funcional por meio de abordagens de mutagênese dirigida. No entanto, este estudo revelou-se essencial para filtrar as 141 mutações encontradas em casos clínicos reais, fornecendo um ponto de partida crucial para proceder a ensaios funcionais com as que se consideram mais relevantes.

Finalmente, estruturas secundárias do RNA nas UTRs foram reconstruídas a partir de estruturas de energia livre mínima e probabilidades no emparelhamento de bases no RNA de fita simples. A predição dessas estruturas secundárias esteve de acordo com as topologias descritas por outros autores (Zeng et al., 1998; Filomatori et al., 2006; Lodeiro et al., 2009; de Borba et al., 2015; Villordo et al., 2015), permitindo assim avaliar o possível efeito das mutações mapeadas nelas. Embora esses resultados não deem uma visão clara dos possíveis distúrbios funcionais causados, resultou curioso que a maioria das variações foram achadas nas estruturas secundárias duplicadas e principalmente em casos de maior gravidade. Pesquisas anteriores sugeriram que a maior variabilidade é comumente detectada em SLII devido a mutações associadas aos mosquitos, e que a duplicação destas estruturas do RNA, ou seja, SLI+SLII e DB1+DB2, permite que o vírus possa se replicar nas células de mamíferos, apesar da presença de

mutações em SLII, que de outra maneira seriam prejudiciais (Villordo et al., 2015). Se este cenário se extrapolasse realmente para o sistema “in vivo”, então as variantes detectadas em SLII e DB2 não deveriam alterar a viabilidade viral em hospedeiros humanos, apesar de causar alterações estruturais. Foi proposto que, manter cópias duplas das estruturas de RNA é uma estratégia viral para garantir a funcionalidade de um elemento conservado, enquanto o outro sofre diferentes pressões seletivas nos dois hospedeiros (Filomatori et al, 2017). Por outro lado, foi demonstrado que SLII atua como um determinante para regular a produção de RNA subgenômico de flavivírus (sfRNA). Esses RNAs curtos não codificantes são produtos da degradação incompleta do genoma viral pela exonuclease Xrn1 nos hospedeiros humanos e mosquitos, e são relevantes na patogênese viral e na evasão da resposta imune. Curiosamente, os vírus que infectam células de mosquito geram um padrão diferente de sfRNAs do que aqueles que infectam células humanas, fenômeno que foi atribuído a mutações específicas em SLII, e levou ao acúmulo de espécies mais curtas de sfRNA (sfRNA3 e sfRNA4) durante a infecção das primeiras. Ao infectar células humanas, esses vírus adaptados a mosquitos exibiram baixo *fitness* induzindo respostas de INF-I mais altas e foram rapidamente substituídos por vírus que geram o sfRNA1 de maior comprimento (Filomatori et al., 2017). Mesmo que a evidência possa indicar que as variantes SLII detectadas aqui possam provavelmente ter surgido no hospedeiro invertebrado e superar os gargalos da transmissão, seu potencial menor *fitness* no hospedeiro humano seria talvez contraintuitivo com o fato de que foram encontradas principalmente em casos de WS+SD. Ensaios funcionais seriam fundamentais para elucidar seu efeito natural na infecção viral.

De forma geral, determinar numa seguinte etapa o efeito das variantes consideradas de maior relevância no *fitness* viral, com a concomitante implementação de mapas de genótipo vs. fenótipo (conhecidos no inglês como “fitness landscapes”) ajudaria a monitorar a evolução deste vírus, e potencialmente, prever trajetórias futuras em direção à virulência e/ou patogenicidade, ou para longe dela. Paralelamente, a confirmação do efeito destas variantes, permitirá que as mesmas sejam contempladas no desenho inteligente de compostos terapêuticos ou profiláticos e na melhoria dos ensaios de diagnóstico.

Em último lugar, é importante destacar que mesmo sendo a base fisiopatológica da dengue um fenômeno multifatorial que depende do equilíbrio

entre os antecedentes genéticos e imunológicos do hospedeiro e os fatores virais, as novas informações sobre as implicações da diversidade genética intra-hospedeiro do DENV-2 que aporte este trabalho, contribuem para o conhecimento dos fatores virais possivelmente envolvidos na sua patogênese no hospedeiro humano, a partir do qual possa-se num futuro sentar as bases para adotar novas medidas que favoreçam o controle e tratamento desta doença.

# 6 CONSLUSÕES

Consistentemente com os três eixos trabalhados ao longo deste projeto, pode-se concluir que:

1a) A cepa viral detectada no estado do Rio de Janeiro em 2019 pertence ao mesmo clado IV que a linhagem de DENV-2 que causou o surto em 2008, no entanto, apresentou um agrupamento filogenético distinto, num cluster totalmente separado das sequências do surto de 2008, porém conjuntamente com sequências de Porto Rico, país que teria dado origem ao ancestral da cepa circulante no Brasil em 2019. Estudos posteriores classificaram esta nova cepa de linhagem BR4 e confirmaram uma possível origem dela no Caribe.

1b) Embora a BR4 possa ser uma nova linhagem se espalhando pelo Brasil, a mesma não resultou tão distante geneticamente da BR3, a diferença do observado entre BR1-BR3. Conjuntamente com o padrão clinico-epidemiológico similar ao desenvolvido por BR3, estas evidencias permitiram a inclusão desta nova cepa no estudo da diversidade intra-hospedeiro de DENV-2.

2a) Pela primeira vez na literatura referente ao DENV, uma abordagem de sequenciamento massivo livre de amplificação por PCR, permitiu refletir de forma fiel a diversidade genética viral em diferentes apresentações clínicas da infecção.

2b) A estrutura da população viral intra-hospedeiro nas amostras estudadas variou de acordo com o tempo de infecção, e em consequência, indiretamente com a gravidade da doença causada pelo DENV-2, uma vez que os casos de maior evolução eram principalmente casos de maior gravidade.

2c) Os casos de DF apresentaram maiores proporções de variantes não sinônimas e indels do que WS e SD, aumentando assim a chance de mutações não sinônimas prejudiciais, proteínas truncadas e partículas virais defeituosas.

2d) As frequências menores apresentadas pelas variantes intra-hospedeiro nos

casos de DF, conjuntamente com os tempos de evolução mais curtos do que WS e SD no processo da infecção, reforçam a hipótese acerca das diferenças observadas entre os grupos clínicos como consequência dos distintos tempos de evolução intra-hospedeiro.

2e) O gene NS2B apresentou uma baixa proporção de variantes intra-hospedeiro não sinônimas quando comparada com os genes restantes, independentemente da clínica dos pacientes. Isto é interpretado como uma baixa tolerância à mutagênese na proteína viral, convertendo-a num alvo atrativo para o desenho de fármacos.

2f) Ao menos nesta amostragem, não se detectou correlação alguma entre a classificação da resposta imune (casos primários ou secundários) e os resultados clínicos dos pacientes.

2g) Não foram evidenciadas diferenças significativas na carga viral e na variabilidade gênica entre os casos primários e secundários deste estudo. No entanto, as infecções secundárias mostraram uma proporção maior de variantes intra-hospedeiro não sinônimas do que as primárias, consistente com seus tempos de infecção mais curtos e, presumivelmente, com as respostas dirigidas.

3a) Abordagens de modelagem molecular por homologia resultaram úteis ferramentas para construir modelos 3D para as proteínas estruturais (C, prM e E) e três das proteínas não estruturais (NS1, NS3 e NS5). No entanto, para as proteínas virais NS2A, NS2B, NS4A e NS4B naturalmente inseridas na membrana do reticulo endoplasmico, nem mediante abordagens de reconhecimento de enovelado nem “de novo” conseguimos obter modelos apropriados que suprissem os mínimos parâmetros de qualidade necessários para o seu posterior uso no estudo das diferentes mutações.

3b) De maneira geral, as variantes disruptivas foram identificadas principalmente entre os casos de DF, enquanto potenciais variantes de escape imunológico foram associados principalmente aos casos de WS+SD, o que está em linha com os tempos de evolução intra-hospedeiro mais longos destes últimos.

3c) Dentre as 141 mutações analisadas, nenhuma aparentou estar relacionada com uma maior virulência ou patogenicidade. As evidências sugerem pelo contrário, que as mutações identificadas entre os casos de WS+SD seriam apenas o resultado da superação dos gargalos seletivos impostos pelas próprias barreiras do hospedeiro humano.

3d) As variantes achadas nas estruturas secundárias duplicadas SLII e DB2, e principalmente entre os casos de maior gravidade, surgiram provavelmente no hospedeiro invertebrado e superaram os gargalos da transmissão, sem causar maiores distúrbios na viabilidade viral nos hospedeiros humanos. No entanto, podem ser determinantes para regular a produção de RNA subgenômico de flavivírus (sfRNA), o qual sabe-se que tem um papel na patogênese viral e na evasão da resposta imune. Frente a esta possibilidade, novos estudos resultam necessários para elucidar se estas mutações têm um efeito direto na gravidade da doença.

# 7 PERSPECTIVAS

Diante todo o exposto nas seções anteriores, resulta evidente que várias linhas de pesquisa ainda podem e deveriam ser exploradas. As mesmas serão brevemente abordadas em seguida.

1. Infecções experimentais em mosquitos empregando amostras de pacientes já sequenciadas por NGS e incluídas neste estudo, com a posterior recuperação das partículas virais permitiriam avaliar se certas variantes virais aqui achadas no nível intra-hospedeiro conseguem efetivamente superar as barreiras de transmissão entre os humanos e os mosquitos, conforme a hipótese levantada anteriormente. Nesse sentido, a identificação de variantes menores com potencial patogênico e, por sua vez, com competência para serem transmitidos e dominar os espectros de mutantes, poderia ser um ponto de partida fundamental para melhorar as vacinas de DENV e as estratégias de resposta frente a surtos de DENV de alto impacto.

2. Resulta essencial desenvolver estudos funcionais que permitam avaliar o efeito das mutações relevantes mencionadas na seção anterior. Sendo assim, uma abordagem eficaz seria a construção de clones portadores das mesmas, de maneira individual. Assim, o efeito no *fitness* viral de cada uma delas poderia em seguida se avaliar tanto em modelos *in vitro* quanto *in vivo*, sem a interferência das restantes variantes presentes na população viral.

3. Poder contar com modelos tridimensionais para as proteínas NS2A, NS2B, NS4A e NS4B seria um excelente ponto de partida para poder testar alvos terapêuticos, entre outros, visto que por exemplo, no caso particular de NS2B foi observada uma baixa tolerância à mutagênese. Logo, a baixa variabilidade desta proteína resulta uma importante vantagem para garantir a eficácia de qualquer fármaco desenhado. As restantes proteínas são associadas com críticas funções tanto na

montagem do complexo de replicação quanto na imunomodulação da resposta à infeção. Sendo assim, resulta fundamental contribuir ao entendimento de como elas atuam para ganhar conhecimento útil para o controle da doença.

4. Embora tenha se considerado cada variante individualmente, seria chave desenvolver alguma estratégia para avaliar os efeitos epistáticos que possam resultar do efeito combinado da presença de várias das mutações sob estudo no mesmo genoma viral, visto que já existem algumas poucas evidencias que demostraram nitidamente como este efeito favoreceu a transmissão do DENV e até se associou a um fenótipo viral de tipo epidêmico (Bellone et al., 2020; Syenina et al., 2020).

5. Conforme descrito anteriormente, o uso de códons na célula do hospedeiro pode ter um impacto considerável na robustez mutacional e em última instancia no *fitness* e capacidade de evolução viral. Vários estudos demostraram também que re-dirigindo o uso de códons ou forçando a mutagênese viral podem ser criados modelos de vacinas atenuadas prometedoras (Lauring et al., 2012; Moratorio et al., 2017). Desta forma, é preciso aprofundar na análise do uso de códons em relação às mutações achadas neste estudo, visando aproveitar essa informação em possíveis novos desenhos para vacinas de DENV.

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**ANEXO I - Registro do projeto no SISNEP e aprovação pelo Comitê de Ética em Pesquisa; CAAE número 90249219.6.1001.5248, Parecer 2.998.362.**

FUNDAÇÃO OSWALDO CRUZ - FIOCRUZ/IOC

## PARECER CONSUBSTANCIADO DO CEP

**DADOS DO PROJETO DE PESQUISA**

**Título da Pesquisa:** ZIKA, DENGUE, CHIKUNGUNYA, FEBRE AMARELA E OUTRAS ARBOVIROSES DE IMPORTÂNCIA CLÍNICO-EPIDEMIOLÓGICA NO BRASIL

**Pesquisador:** Ana Maria Bispo de Filippis

**Área Temática:**

**Versão:** 2

**CAAE:** 90249218.6.1001.5248

**Instituição Proponente:** Instituto Oswaldo Cruz-RJ

**Patrocinador Principal:** The Wellcome Trust
FUN CARLOS CHAGAS F. DE AMPARO A PESQUISA DO ESTADO DO RIO DE JANEIRO - FAPERJ
World Health Organization
Ministério da Saúde

**DADOS DO PARECER**

**Número do Parecer:** 2.998.362

**Considerações Finais a critério do CEP:**

Diante do exposto, o Comitê de Ética em Pesquisa do Instituto Oswaldo Cruz (CEP Fiocruz/IOC), em sua 243ª Reunião Extraordinária, de acordo com as atribuições definidas na Resolução CNS nº 510 de 2016, na Resolução CNS 466/12 e na Norma Operacional nº 001 de 2013 do CNS, manifesta-se por APROVAR o projeto em tela, mesmo na ausência da tradução para o português da carta da Rush University. Favor enviar a mesma, via Plataforma Brasil sob a forma de Notificação.

**Endereço:** Av. Brasil 4036, Sala 705 (Campus Expansão)
**Bairro:** Manguinhos **CEP:** 21.040-360
**UF:** RJ **Município:** RIO DE JANEIRO
**Telefone:** (21)3882-9011 **Fax:** (21)2561-4815 **E-mail:** cepfiocruz@ioc.fiocruz.br

**ANEXO II - Características das amostras coletadas para o desenvolvimento deste projeto.** Destacadas em vermelho, as amostras que conformaram o grupo para o estudo da diversidade intra-hospedeiro. MG: Estado de Minas Gerais; RJ: Estado do Rio de Janeiro; SP: Estado de São Paulo; CT: número de ciclos em no ensaio RT-PCR necessário para amplificar o RNA viral acima do limite de detecção, do inglês “cycle treshold”; ufp/ml: unidades formadoras de placa por mililitro; CV: carga viral; CC: concentração; ng/ul: nanograma/microlitro; nM: concentração nanomolar; NGS: sequenciamento de nova geração, do inglês “Next Generation Sequencing”; DF: dengue clássico, do inglês “dengue fever”; WS: dengue com sinais de alarme, do inglês “dengue with warning signs”; SD: dengue grave, do inglês “severe dengue”; H: homem; M: mulher.

| Amostra | Data de coleta | Dias de sintomas | Clasificação clínica | Género | Idade | Estado/Municipio | CT | Carga Viral (ufp/ml) | CV pós depleção+síntese do cDNA (ufp/ml) | Index | CC Qubit (ng/ul) | CC estimada Bioanalyzer (nM) | Sequenciamento |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 160 | 28/04/2019 | 0 | DF | M | 24 | MG/Belo Horizonte | 18.98 | 9.97E+05 | 1.47E+04 | 1A | 9.1 | 73.3 | NGS |
| 161 | 08/05/2019 | 3 | DF | M | 28 | MG/Belo Horizonte | 20.01 | 5.31E+05 | 9.49E+03 | 1C | 8.82 | 61.3 | NGS |
| 162 | 02/05/2019 | 2 | DF | H | 44 | MG/Luz | 20.63 | 3.64E+05 | 1.08E+04 | 1F | 4.66 | 27.4 | NGS |
| 163 | 02/05/2019 | 2 | DF | M | 28 | MG/Belo Horizonte | 20.69 | 3.51E+05 | 8.64E+03 | 1G | 7.18 | 46.3 | NGS |
| 164 | 12/04/2019 | 3 | DF | M | 47 | MG/Betim | 24.31 | 3.83E+04 | 2.11E+03 | 9D | 3.04 | 19 | NGS - Baixa qualidade |
| 165 | 01/03/2019 | 0 | DF | H | 26 | RJ/Nova Iguaçu | 23.59 | 9.98E+03 | 2.43E+03 | 9C | 0.43 | 4.14 | NGS - Baixa qualidade |
| 166 | 04/04/2019 | 1 | DF | H | 65 | MG/Sete Lagoas | 20.02 | 5.28E+05 | 7.07E+04 | 2G | 9.24 | 56.5 | NGS |
| 167 | 04/04/2019 | 3 | DF | M | 51 | MG/Tres Pontas | 20.18 | 4.79E+05 | 1.95E+03 | 2C | 4.1 | 30.8 | NGS |
| 168 | 26/04/2019 | 2 | DF | H | 53 | MG/Bom Despacho | 21.07 | 2.78E+05 | 2.57E+04 | 2D | 8.82 | 54.7 | NGS |
| 169 | 26/04/2019 | 2 | DF | M | 30 | MG/Belo Horizonte | 21.37 | 2.31E+05 | 3.29E+04 | 2E | 11.3 | 65.2 | NGS |
| 170 | 26/04/2019 | 1 | DF | M | 26 | MG/Belo Horizonte | 23.82 | 5.17E+04 | 4.75E+04 | 2F | 8.5 | 48.5 | NGS |
| 171 | 08/05/2019 | 1 | DF | M | 49 | MG/Sete Lagoas | 18.48 | 1.35E+06 | 3.53E+04 | 2H | 5.12 | 26.2 | NGS |
| 172 | 09/04/2019 | 2 | DF | M | 65 | MG/Araxa | 15.5 | 8.38E+06 | 7.28E+06 | 3H | 5.72 | 31.6 | NGS |
| 173 | 29/01/2019 | 4 | DF | H | 42 | RJ/Vassouras | 22.31 | 2.82E+04 | 5.35E+03 | 5A | 1.25 | 8.4 | NGS |
| 174 | 22/02/2019 | 3 | DF | H | 59 | RJ/Volta Redonda | 22.37 | 2.69E+04 | 3.05E+03 | 5B | 1.62 | 9.4 | NGS |
| 175 | 11/03/2019 | 1 | DF | H | 37 | RJ/Volta Redonda | 21.84 | 4.13E+04 | 5.12E+03 | 5C | 2.66 | 16.3 | NGS |
| 176 | 07/04/2018 | 1 | DF | M | 64 | RJ/Rio de Janeiro | 21.88 | 4.00E+04 | 1.52E+03 | 5D | 3.26 | 16.5 | NGS |
| 177 | 20/05/2019 | 4 | DF | H | 40 | RJ/Volta Redonda | 22.16 | 3.18E+04 | 8.85E+03 | 5E | 2.82 | 12.5 | NGS |
| 178 | 20/05/2019 | 4 | DF | H | 36 | RJ/Volta Redonda | 21.61 | 4.98E+04 | 9.72E+03 | 5F | 1.68 | 6.5 | NGS |
| 179 | 22/05/2019 | 0 | DF | M | 44 | RJ/Vassouras | 22.08 | 3.40E+04 | 9.31E+03 | 5G | 2.52 | 14.4 | NGS |
| 180 | 03/06/2019 | 2 | DF | M | 28 | RJ/Parati | 22.2 | 3.08E+04 | 1.28E+04 | 5H | 2.08 | 14.2 | NGS |
| 181 | 25/04/2019 | 2 | DF | M | 21 | MG/Belo Horizonte | 23.14 | 7.84E+04 | 8.56E+02 | 6D | 0.63 | 3.9 | NGS |
| 182 | 03/05/2019 | 2 | DF | M | 19 | MG/Araxa | 24.15 | 4.23E+04 | 8.37E+02 | 6E | 2.7 | 14.8 | NGS |
| 183 | 02/04/2019 | 1 | DF | H | 59 | MG/Betim | 21.36 | 2.33E+05 | 9.55E+03 | 6F | 2.1 | 12.1 | NGS |
| 184 | 15/04/2019 | 0 | DF | M | 54 | MG/Arceburgo | 24.54 | 3.33E+04 | 7.98E+02 | 6H | 2.14 | 12.9 | NGS |
| 185 | 09/03/2010 | 2 | DWS | M | 31 | RJ/Campos dos Goytacazes | 18.45 | 6.47E+05 | 1.02E+05 | 3A | 2.6 | 15.2 | NGS |
| 186 | 09/03/2010 | 3 | DWS | H | 13 | RJ/Campos dos Goytacazes | 22.99 | 1.62E+04 | 3.02E+03 | 3B | 1.33 | 6.98 | NGS |
| 187 | 26/01/2011 | 3 | DWS | M | 24 | RJ/Campos dos Goytacazes | 21.34 | 6.19E+04 | 1.17E+04 | 3C | 1.71 | 8.3 | NGS |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 188 | 16/02/2011 | 2 | DWS | H | 31 | RJ/Campos dos Goytacazes | 17.57 | 1.32E+06 | 1.32E+05 | 3D | 1.76 | 7.1 | NGS |
| 189 | 30/01/2010 | 1 | DWS | H | 74 | RJ/Campos dos Goytacazes | 22.43 | 1.21E+05 | 6.98E+03 | 3E | 1.5 | 6.5 | NGS |
| 190 | 19/03/2019 | 2 | DWS | H | 57 | MG/Araxa | 22.76 | 9.89E+04 | 6.64E+03 | 3F | 2.48 | 15.7 | NGS |
| 191 | 16/09/2009 | 0 | DWS | H | 54 | RJ/Rio de Janeiro | 28.1 | 3.78E+03 | 7.27E+02 | 4B | 10.9 | 47.5 | NGS |
| 192 | 16/02/2011 | 2 | DWS | H | 36 | RJ/Campos dos Goytacazes | 23.97 | 7.33E+03 | 6.84E+03 | 4D | 0.86 | 6.8 | NGS |
| 193 | 08/05/2019 | 3 | DWS | M | 24 | MG/Belo Horizonte | 24.94 | 2.61E+04 | 1.01E+03 | 9G | 2.94 | 19.1 | NGS |
| 194 | 07/04/2011 | 4 | SD | H | 7m | RJ/São Gonçalo | 28.39 | 3.16E+03 | 2.37E+01 | 7A | 4.66 | 35 | NGS |
| 195 | 18/03/2010 | 4 | SD | H | 86 | SP/Santos | 29 | 7.17E+01 | 5.66E+01 | 7B | 9.68 | 71.5 | NGS |
| 196 | 24/03/2010 | 5 | SD - óbito | M | 64 | SP/Santos | 29.85 | 3.89E+01 | 6.45E+00 | 7C | 1.85 | 10.5 | NGS |
| 197 | 23/04/2008 | 6 | SD | M | - | RJ/Rio de Janeiro | 33.5 | 4.17E+00 | 1.25E+00 | 7D | 8.36 | 32 | NGS |
| 198 | 23/04/2008 | 7 | SD | M | - | RJ/Rio de Janeiro | 30.5 | 3.79E+01 | 3.39E+01 | 7E | 21.2 | 103.8 | NGS |
| 199 | 05/12/2008 | 7 | SD - óbito | H | 7 | RJ/Duque de Caxias | 28.26 | 2.25E+02 | 2.21E+00 | 8B | 2.38 | 12.5 | NGS |
| 200 | 23/04/2008 | 4 | SD - óbito | M | 44 | RJ/Rio de Janeiro | 30.93 | 6.69E+02 | 3.23E+00 | 8D | 3.02 | 14.2 | NGS |
| 201 | 08/02/2008 | 4 | SD | F | 9 | RJ/Rio de Janeiro | 33.49 | 3.23E+00 | 1.20E+00 | 11A | 3.12 | 17.9 | NGS |
| 202 | 04/04/2010 | - | SD - óbito | H | 2 | SP/Santos | 20.4 | 3.45E+04 | 4.75E+04 | 3G | 1.21 | 8 | NGS |
| 203 | 16/03/2008 | 5 | SD | H | 88 | RJ/Rio de Janeiro | 27.72 | 4.76E+03 | 3.08E+02 | 4E | 1.4 | 7.4 | NGS |
| 204 | 12/03/2010 | 8 | SD - óbito | M | 50 | RJ/Rio de Janeiro | 27.15 | 6.75E+03 | 5.87E+02 | 4C | 0.78 | 4.86 | NGS |
| 205 | 18/01/2010 | 1 | DWS | H | 19 | RJ/Campos dos Goytacazes | 35.6 | 3.85E+01 | 3.75E-01 | 11D | 0.97 | 5.7 | NGS |
| 206 | 02/03/2010 | 5 | DWS | H | 21 | SP/Santos | 32.7 | 5.03E+00 | 8.00E-01 | 11E | 2.48 | 13.2 | NGS |
| 207 | 17/03/2009 | 5 | DWS/SD? | H | 60 | RJ/Niteroi | 32 | 1.08E+01 | 7.51E-01 | 11C | 5.94 | 21.8 | NGS |
| 208 | 07/02/2008 | 24! | SD - óbito | H | 15 | RJ/Rio de Janeiro | 33.64 | 2.86E+00 | 2.52E-01 | 7F | 5.4 | 31 | NGS |
| 209 | 22/03/2010 | 6 | SD - óbito | M | 12 | SP/Santos | 32.14 | 7.52E+00 | 5.09E-01 | 11B | 2.68 | 16.7 | NGS |
| 210 | 09/05/2019 | 2 | DF | M | 26 | MG/Belo Horizonte | 24.28 | 3.90E+04 | 4.91E+02 | 1D | 8.16 | 56.4 | NGS - Baixa qualidade |
| 211 | 09/05/2019 | 1 | DF | M | 39 | MG/Gobernador Valadares | 22.52 | 1.15E+05 | 1.83E+03 | 1E | 9.5 | 67.3 | NGS - Baixa qualidade |
| 212 | 11/05/2019 | 3 | DF | H | 39 | MG/Varginha | 21.97 | 1.60E+05 | 2.84E+03 | 1H | 42.4 | 382.4 | NGS - Baixa qualidade |
| 213 | 25/04/2019 | 3 | DF | H | 40 | MG/Varzelandia | 24.62 | 3.17E+04 | 1.04E+03 | 6G | 2.96 | 17.6 | NGS - Baixa qualidade |
| 214 | 12/04/2019 | 3 | DF | M | 68 | MG/Araguari | 20.72 | 3.44E+05 | 1.37E+05 | 2A | Muito baixo | - | - |
| 215 | 16/04/2019 | 0 | DF | M | 68 | MG/Perdoes | 19.07 | 9.44E+05 | 5.57E+04 | 2B | Muito baixo | - | - |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 216 | 10/04/2019 | 2 | DF | M | 37 | MG/Uberaba | 22.85 | 9.36E+04 | 8.90E+02 | 6A | Muito baixo | - | - |
| 217 | 17/04/2019 | 1 | DF | M | 33 | MG/Belo Horizonte | 23.11 | 7.98E+04 | 8.97E+02 | 6B | Muito baixo | - | - |
| 218 | 22/04/2019 | 0 | DF | M | 32 | MG/Belo Horizonte | 20.22 | 4.67E+05 | 1.14E+04 | 6C | Muito baixo | - | - |
| 219 | 26/03/2010 | 3 | DF | H | 61 | SP/Santos | 24.92 | 3.39E+03 | 1.80E+03 | 9A | Muito baixo | - | - |
| 220 | 09/02/2019 | 3 | DF | M | 26 | RJ/Vassouras | 25.94 | 1.48E+03 | 6.91E+02 | 9B | Muito baixo | - | - |
| 221 | 05/03/2010 | 4 | SD | M | 12 | SP/Santos | 33.9 | 2.13E+00 | Neg. | - | - | - | - |
| 222 | 22/03/2010 | 8 | DWS/SD? | H | 47 | SP/Santos | 34.7 | 1.20E+00 | Neg. | - | - | - | - |
| 223 | 11/03/2008 | 7 | DWS/SD? | H | 10 | RJ/Rio de Janeiro | 35.6 | 3.85E+01 | Neg. | - | - | - | - |
| 224 | 23/04/2008 | 6 | SD | H | - | RJ/Rio de Janeiro | 34.7 | 1.20E+00 | Neg. | - | - | - | - |
| 225 | 30/03/2010 | 8 | DWS | H | 24 | SP/Santos | 34.61 | 1.30E+00 | Neg. | - | - | - | - |
| 226 | 14/04/2010 | 5 | DWS | M | 39 | SP/Santos | 34.57 | 1.34E+00 | Neg. | - | - | - | - |
| 227 | 23/04/2008 | 7 | DF | M | | RJ/Rio de Janeiro | 34.63 | 1.37E+00 | - | - | - | - | - |
| 228 | 14/01/2019 | 3 | DF | M | 38 | RJ/Vassouras | 26.49 | 9.48E+02 | - | - | - | - | - |
| 229 | 21/01/2019 | 3 | DF | H | 60 | RJ/Vassouras | 33.47 | 3.28E+00 | - | - | - | - | - |
| 230 | 20/05/2019 | 2 | DF | M | 53 | RJ/Volta Redonda | 28.51 | 1.84E+02 | - | - | - | - | - |
| 231 | 16/05/2019 | 1 | DF | H | 31 | RJ/Cabo Frio | 26.68 | 8.12E+02 | - | - | - | - | - |
| 232 | 15/05/2019 | 4 | DF | H | 22 | RJ/Porciuncula | 28.62 | 1.68E+02 | - | - | - | - | - |
| 233 | 15/05/2019 | 4 | DF | M | 26 | RJ/Porciuncula | 27.07 | 5.92E+02 | - | - | - | - | - |
| 234 | 24/05/2019 | 3 | DF | M | 64 | RJ/Cabo Frio | 26.71 | 7.93E+02 | - | - | - | - | - |
| 235 | 07/06/2019 | 3 | DF | H | 23 | RJ/Cabo Frio | 29.21 | 1.04E+02 | - | - | - | - | - |
| 236 | 19/06/2019 | 1 | DF | H | 29 | RJ/Pirai | 31.05 | 2.34E+01 | - | - | - | - | - |
| 237 | 12/04/2019 | 3 | DF | M | 52 | MG/Betim | 21.87 | 1.70E+05 | - | - | - | - | - |
| 238 | 03/04/2019 | 0 | DF | M | 47 | MG/Tres Pontas | 19.77 | 6.15E+05 | - | - | - | - | - |
| 239 | 29/03/2019 | 1 | DF | H | 8 | MG/Araxa | 19.18 | 8.83E+05 | - | - | - | - | - |
| 240 | 28/03/2019 | 3 | DF | M | 15 | MG/Araxa | 21.67 | 1.93E+05 | - | - | - | - | - |
| 241 | 05/04/2019 | 3 | DF | M | 38 | MG/Verissimo | 27.44 | 5.65E+03 | - | - | - | - | - |
| 242 | 05/04/2019 | 2 | DF | M | 37 | MG/Verissimo | 22.53 | 1.14E+05 | - | - | - | - | - |
| 243 | 05/04/2019 | 1 | DF | M | 48 | MG/Verissimo | 18.82 | 1.10E+06 | - | - | - | - | - |
| 244 | 05/04/2019 | 4 | DF | M | 44 | MG/Verissimo | 19.49 | 7.30E+05 | - | - | - | - | - |
| 245 | 18/03/2019 | 3 | DF | M | 62 | MG/Araxa | 18.37 | 1.45E+06 | - | - | - | - | - |

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| 246 | 08/04/2019 | 2 | DF | M | 30 | MG/Araxa | 24.94 | 2.61E+04 | - | - | - | - | - |
| 247 | 07/04/2019 | 1 | DF | M | 23 | MG/Verissimo | 23.51 | 6.25E+04 | - | - | - | - | - |
| 248 | 07/04/2019 | 3 | DF | M | 25 | MG/verissimo | 25.88 | 1.47E+04 | - | - | - | - | - |
| 249 | 25/03/2019 | 4 | DF | M | 62 | MG/Itapagipe | 21.68 | 1.91E+05 | - | - | - | - | - |
| 250 | 14/04/2019 | 3 | DF | H | 62 | MG/Itapagipe | 21.95 | 1.62E+05 | - | - | - | - | - |
| 251 | 16/04/2019 | 3 | DF | M | 67 | MG/Itapagipe | 24.44 | 3.54E+04 | - | - | - | - | - |
| 252 | 08/04/2019 | 3 | DF | H | 71 | MG/Bom Despacho | 24.2 | 4.10E+04 | - | - | - | - | - |
| 253 | 09/04/2019 | 1 | DF | H | 65 | MG/Arceburgo | 21.42 | 2.24E+05 | - | - | - | - | - |
| 254 | 09/04/2019 | 2 | DF | M | 49 | MG/Monjolos | 19.1 | 9.27E+05 | - | - | - | - | - |
| 255 | 10/04/2019 | 2 | DF | M | 47 | MG/Pompeu | 16.75 | 3.90E+06 | - | - | - | - | - |
| 256 | 11/04/2019 | 2 | DF | M | 30 | MG/Pompeu | 25.77 | 1.57E+04 | - | - | - | - | - |
| 257 | 25/04/2019 | 2 | DF | M | 40 | MG/Varginha | 23.46 | 6.44E+04 | - | - | - | - | - |
| 258 | 24/04/2019 | 2 | DF | M | 25 | MG/Bom Despacho | 19.57 | 6.95E+05 | - | - | - | - | - |
| 259 | 25/04/2019 | 3 | DF | M | 20 | MG/Bom Despacho | 23.97 | 4.72E+04 | - | - | - | - | - |
| 260 | 02/05/2019 | 3 | DF | H | 26 | MG/Luz | 26.67 | 9.05E+03 | - | - | - | - | - |
| 261 | 15/04/2019 | 3 | DF | M | 30 | MG/Sao Gonçalo do Para | 22.06 | 1.52E+05 | - | - | - | - | - |
| 262 | 15/04/2019 | 3 | DF | M | 21 | MG/Sao Gonçalo do Para | 18.67 | 1.21E+06 | - | - | - | - | - |
| 263 | 07/05/2019 | 4 | DF | H | 16 | MG/Ponte Nova | 21.53 | 2.10E+05 | - | - | - | - | - |
| 264 | 01/05/2019 | 2 | DF | H | 53 | MG/Varginha | 20.48 | 3.99E+05 | - | - | - | - | - |
| 265 | 03/05/2019 | 2 | DF | M | 23 | MG/Varginha | 20.5 | 3.94E+05 | - | - | - | - | - |
| 266 | 30/04/2019 | 3 | DF | H | 36 | MG/Varginha | 24.82 | 2.81E+04 | - | - | - | - | - |
| 267 | 02/04/2019 | 2 | DF | H | 32 | MG/Sete Lagoas | 20.01 | 5.31E+05 | - | - | - | - | - |
| 268 | 03/04/2019 | 2 | DF | H | 29 | MG/Sete Lagoas | 22.83 | 9.47E+04 | - | - | - | - | - |
| 269 | 02/04/2019 | 2 | DF | M | 18 | MG/Sete Lagoas | 23.03 | 8.38E+04 | - | - | - | - | - |
| 270 | 29/03/2010 | ND | DF | M | 12 | SP/Santos | 33.55 | 3.08E+00 | - | - | - | - | - |
| 271 | 23/04/2008 | 6 | Grave | H | | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 272 | 23/04/2008 | ND | Grave | H | | RJ/Rio de Janeiro | 36.8 | 2.65E-01 | - | - | - | - | - |
| 273 | 23/04/2008 | 9 | Grave | M | | RJ/Rio de Janeiro | 36.8 | 2.65E-01 | - | - | - | - | - |
| 274 | 23/04/2008 | 8 | Grave | H | | RJ/Rio de Janeiro | 37.3 | 1.85E-01 | - | - | - | - | - |
| 275 | 01/05/2008 | 5 | Grave | M | | RJ/Rio de Janeiro | 35.6 | 6.27E-01 | - | - | - | - | - |
| 276 | 22/04/2008 | 11 | Grave | H | | RJ/Rio de Janeiro | 37.04 | 2.23E-01 | - | - | - | - | - |
| 277 | 22/04/2008 | 13 | Clásico | M | | RJ/Rio de Janeiro | 36.8 | 2.65E-01 | - | - | - | - | - |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 278 | 23/04/2008 | 14 | Clásico | M | RJ/Rio de Janeiro | 38.03 | 8.11E-02 | - | - | - | - | - |
| 279 | 23/04/2008 | 10 | Clásico | M | RJ/Rio de Janeiro | 37.8 | 1.29E-01 | - | - | - | - | - |
| 280 | 23/04/2008 | 9 | Grave | H | RJ/Rio de Janeiro | 38.9 | 5.86E-02 | - | - | - | - | - |
| 281 | 23/04/2008 | 9 | Grave | M | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 282 | 29/04/2008 | 11 | Grave | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 283 | 29/04/2008 | 11 | Grave | M | RJ/Rio de Janeiro | 36.7 | 2.85E-01 | - | - | - | - | - |
| 284 | 01/05/2008 | 13 | Grave | M | RJ/Rio de Janeiro | 36.8 | 2.65E-01 | - | - | - | - | - |
| 285 | 01/05/2008 | 10 | Grave | M | RJ/Rio de Janeiro | 38.6 | 7.27E-02 | - | - | - | - | - |
| 286 | 24/04/2008 | 10 | Grave | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 287 | 24/04/2008 | 8 | Grave | H | RJ/Rio de Janeiro | 38.9 | 5.86E-02 | - | - | - | - | - |
| 288 | 25/04/2008 | 8 | Grave | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 289 | 26/04/2008 | 8 | Grave | M | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 290 | 01/05/2008 | 8 | Grave | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 291 | 02/05/2008 | 9 | Grave | M | RJ/Rio de Janeiro | 38.7 | 6.77E-02 | - | - | - | - | - |
| 292 | 03/05/2008 | 13 | Grave | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 293 | 03/05/2008 | 11 | Grave | H | RJ/Rio de Janeiro | 38.8 | 6.30E-02 | - | - | - | - | - |
| 294 | 08/05/2008 | 6 | Grave | M | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 295 | 12/05/2008 | 11 | Grave | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 296 | 13/05/2008 | 10 | Grave | M | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 297 | 13/05/2008 | 11 | Grave | M | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 298 | 14/05/2008 | 7 | Grave | M | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 299 | 19/04/2008 | 10 | Grave | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 300 | 14/05/2008 | 8 | Grave | H | RJ/Rio de Janeiro | 37.26 | 1.90E-01 | - | - | - | - | - |
| 301 | 21/04/2008 | 11 | Clásico | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 302 | 22/04/2008 | 12 | Clásico | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 303 | 22/04/2008 | 10 | Clásico | H | RJ/Rio de Janeiro | 36 | 4.70E-01 | - | - | - | - | - |
| 304 | 22/04/2008 | 10 | Clásico | H | RJ/Rio de Janeiro | 38.75 | 6.53E-02 | - | - | - | - | - |
| 305 | 22/04/2008 | 8 | Clásico | M | RJ/Rio de Janeiro | 36.9 | 2.47E-01 | - | - | - | - | - |
| 306 | 23/04/2008 | 7 | Clásico | M | RJ/Rio de Janeiro | 37.3 | 1.85E-01 | - | - | - | - | - |
| 307 | 23/04/2008 | - | Clásico | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 308 | 23/04/2008 | 8 | Clásico | H | RJ/Rio de Janeiro | 35 | 9.65E-01 | - | - | - | - | - |
| 309 | 23/04/2008 | 9 | Clásico | M | RJ/Rio de Janeiro | 37.7 | 1.39E-01 | - | - | - | - | - |
| 310 | 29/04/2008 | 11 | Clásico | H | RJ/Rio de Janeiro | 35.3 | 7.78E-01 | - | - | - | - | - |

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| 311 | 29/04/2008 | 12 | Clásico | M | RJ/Rio de Janeiro | 38.2 | 9.69E-02 | - | - | - | - | - |
| 312 | 29/04/2008 | 9 | Clásico | H | RJ/Rio de Janeiro | 38.23 | 9.49E-02 | - | - | - | - | - |
| 313 | 29/04/2008 | 12(+6) | Clásico | M | RJ/Rio de Janeiro | 38.3 | 9.02E-02 | - | - | - | - | - |
| 314 | 29/04/2008 | 11 | Clásico | M | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 315 | 29/04/2008 | 13 | Clásico | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 316 | 29/04/2008 | 9?(-2d) | Clásico | M | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 317 | 01/05/2008 | 11(+2d) | Clásico | M | RJ/Rio de Janeiro | 38.2 | 9.69E-02 | - | - | - | - | - |
| 318 | 29/04/2008 | - | Clásico | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 319 | 01/05/2008 | 8 | Clásico | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 320 | 01/05/2008 | 6 | Clásico | M | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 321 | 01/05/2008 | 12 | Grave | H | RJ/Rio de Janeiro | 36.48 | 3.33E-01 | - | - | - | - | - |
| 322 | 01/05/2008 | 12 | Clásico | M | RJ/Rio de Janeiro | 36.55 | 3.17E-01 | - | - | - | - | - |
| 323 | 01/05/2008 | 11 | Clásico | H | RJ/Rio de Janeiro | 37.15 | 2.06E-01 | - | - | - | - | - |
| 324 | 01/05/2008 | 8 | Clásico | M | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 325 | 25/03/2008 | 4 | Grave - óbito | M | RJ/Duque de Caxias | 35.56 | 6.02E-01 | - | - | - | - | - |
| 326 | 29/01/2019 | 3 | Clásico | M | RJ/Vassouras | 38.3 | 6.51E-02 | - | - | - | - | - |
| 327 | 08/03/2019 | 1 | Clásico | M | RJ/Volta Redonda | Neg. | - | - | - | - | - | - |
| 328 | 25/02/2019 | 0 | Clásico | H | RJ/Mangaratiba | 35.67 | 5.51E-01 | - | - | - | - | - |
| 329 | 07/05/2019 | 3 | - | M | RJ/Tibau do Sul | Neg. | - | - | - | - | - | - |
| 330 | 16/05/2019 | 4 | - | M | RJ/Porciuncula | 36.01 | 4.18E-01 | - | - | - | - | - |
| 331 | 03/03/2019 | 0 | - | H | RJ/Rio de Janeiro | Neg. | - | - | - | - | - | - |
| 332 | 03/06/2019 | 1 | - | M | RJ/Parati | Neg. | - | - | - | - | - | - |
| 333 | 24/02/2010 | 12 | Grave | M | SP/Santos | 36.96 | 2.36E-01 | - | - | - | - | - |
| 334 | 24/02/2010 | 6 | Grave | M | SP/Santos | 35 | 9.65E-01 | - | - | - | - | - |
| 335 | 25/02/2010 | 7 | Grave | H | SP/Santos | 37.1 | 2.14E-01 | - | - | - | - | - |
| 336 | 26/02/2010 | 12 | Grave - óbito | M | SP/Santos | 38.2 | 9.69E-02 | - | - | - | - | - |
| 337 | 15/03/2010 | 9 | Grave | M | SP/Santos | Neg. | - | - | - | - | - | - |
| 338 | 18/03/2010 | 7 | Grave | M | SP/Santos | 35.7 | 5.84E-01 | - | - | - | - | - |
| 339 | 29/03/2010 | 4 | Grave | H | SP/Santos | Neg. | - | - | - | - | - | - |
| 340 | 31/03/2010 | - | Grave | M | SP/Santos | 38 | 1.12E-01 | - | - | - | - | - |
| 341 | 31/03/2010 | 9 | Grave? Ws? | H | SP/Santos | 35.32 | 7.67E-01 | - | - | - | - | - |
| 342 | 06/04/2010 | 5 | Grave - óbito | H | SP/Santos | Neg. | - | - | - | - | - | - |
| 343 | 13/04/2010 | 6 | Grave | H | SP/Santos | 36 | 4.70E-01 | - | - | - | - | - |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 344 | 12/04/2010 | 2 | Grave | H | SP/Santos | 35.4 | 7.24E-01 | - | - | - | - | - |
| 345 | 12/04/2010 | 4 | Grave | M | SP/Santos | 35.8 | 5.43E-01 | - | - | - | - | - |
| 346 | 24/02/2010 | 5 | Sinais de alarme | H | SP/Santos | Neg. | - | - | - | - | - | - |
| 347 | 25/02/2010 | 2 | Sinais de alarme | H | SP/Santos | Neg. | - | - | - | - | - | - |
| 348 | 01/03/2010 | 5 | Sinais de alarme | M | SP/Santos | 38.22 | 6.95E-02 | - | - | - | - | - |
| 349 | 04/03/2010 | 2 | Clásico | M | SP/Santos | 35.12 | 8.61E-01 | - | - | - | - | - |
| 350 | 08/03/2010 | 9 | Clásico | H | SP/Santos | Neg. | - | - | - | - | - | - |
| 351 | 09/03/2010 | 5 | Sinais de alarme? | M | SP/Santos | 37.27 | 1.50E-01 | - | - | - | - | - |
| 352 | 12/03/2010 | 8 | Sinais de alarme | H | SP/Santos | 38.04 | 8.05E-02 | - | - | - | - | - |
| 353 | 12/03/2010 | 9 | Clásico | H | SP/Santos | Neg. | - | - | - | - | - | - |
| 354 | 19/03/2010 | 4 | Clásico | M | SP/Santos | 34.94 | 9.96E-01 | - | - | - | - | - |
| 355 | 22/03/2010 | 8 | Sinais de alarme | H | SP/Santos | 38.35 | 6.26E-02 | - | - | - | - | - |
| 356 | 22/03/2010 | 7 | Sinais de alarme | - | SP/Santos | Neg. | - | - | - | - | - | - |
| 357 | 23/03/2010 | 10 | Sinais de alarme | H | SP/Santos | Neg. | - | - | - | - | - | - |
| 358 | 24/03/2010 | 3 | Clásico | M | SP/Santos | 37.1 | 1.73E-01 | - | - | - | - | - |
| 359 | 25/03/2010 | 4 | Clásico | M | SP/Santos | 38.93 | 3.91E-02 | - | - | - | - | - |
| 360 | 30/03/2010 | 6 | Sinais de alarme | H | SP/Santos | 37.83 | 9.54E-02 | - | - | - | - | - |
| 361 | 27/03/2010 | 2 | Clásico | M | SP/Santos | 36.74 | 2.31E-01 | - | - | - | - | - |
| 362 | 31/03/2010 | 7 | Clásico | H | SP/Santos | Neg. | - | - | - | - | - | - |
| 363 | 01/04/2010 | 7 | Sinais de alarme | M | SP/Santos | Neg. | - | - | - | - | - | - |
| 364 | 09/04/2010 | 8 | Sinais de alarme | M | SP/Santos | 36.18 | 3.64E-01 | - | - | - | - | - |
| 365 | 14/04/2010 | 5 | Sinais de alarme | M | SP/Santos | 36.36 | 3.15E-01 | - | - | - | - | - |
| 366 | 15/04/2010 | 5 | Sinais de alarme | M | SP/Santos | Neg. | - | - | - | - | - | - |